

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|  |           |  |
|--|-----------|--|
| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12N 15/12, C12Q 1/68, A01K 67/00,</b><br><b>A61K 48/00, C07K 14/47</b>   | <b>A2</b> | <b>(11) International Publication Number:</b> <b>WO 95/15381</b><br><b>(43) International Publication Date:</b> 8 June 1995 (08.06.95)   |
| <b>(21) International Application Number:</b> PCT/US94/13805<br><b>(22) International Filing Date:</b> 2 December 1994 (02.12.94)<br><br><b>(30) Priority Data:</b><br>160,295 2 December 1993 (02.12.93) US<br><br><b>(71) Applicant:</b> THE JOHNS HOPKINS UNIVERSITY [US/US];<br>720 Rutland Avenue, Baltimore, MD 21205 (US).<br><br><b>(71)(72) Applicant and Inventor:</b> DE LA CHAPELLE, Albert<br>[FI/FI]; Bregmansgatan 11, FIN-00140 Helsingfors (FI).<br><br><b>(72) Inventors:</b> VOGELSTEIN, Bert; 3700 Breton Way, Baltimore,<br>MD 21208 (US). KINZLER, Kenneth, W.; 1348 Halstead<br>Road, Baltimore, MD 21234 (US).<br><br><b>(74) Agents:</b> KAGAN, Sarah, A. et al.; Banner, Birch, McKie &<br>Beckett, 11th floor, 1001 G Street, N.W., Washington, DC<br>20001 (US). |           | <b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, DE,<br>DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).<br><br><b>Published</b><br><i>Without international search report and to be republished<br/>upon receipt of that report.</i> |
| <b>(54) Title:</b> HUMAN MUTATOR GENE hMSH2 AND HEREDITARY NON POLYPOSIS COLORECTAL CANCER<br><br><b>(57) Abstract</b><br><br>The human <i>MSH2</i> gene, responsible for hereditary non-polyposis colorectal cancer, was identified by virtue of its homology to the <i>MutS</i> class of genes, which are involved in DNA mismatch repair. The sequence of cDNA clones of the human gene are provided, and the sequence of the gene can be used to demonstrate the existence of germ line mutations in hereditary non-polyposis colorectal cancer (HNPCC) kindreds, as well as in replication error <sup>+</sup> (RER <sup>+</sup> ) tumor cells.  |           |  |

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |                                       |    |                          |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria                  | GB | United Kingdom                        | MR | Mauritania               |
| AU | Australia                | GE | Georgia                               | MW | Malawi                   |
| BB | Barbados                 | GN | Guinea                                | NE | Niger                    |
| BE | Belgium                  | GR | Greece                                | NL | Netherlands              |
| BF | Burkina Faso             | HU | Hungary                               | NO | Norway                   |
| BG | Bulgaria                 | IE | Ireland                               | NZ | New Zealand              |
| BJ | Benin                    | IT | Italy                                 | PL | Poland                   |
| BR | Brazil                   | JP | Japan                                 | PT | Portugal                 |
| BY | Belarus                  | KE | Kenya                                 | RO | Romania                  |
| CA | Canada                   | KG | Kyrgyzstan                            | RU | Russian Federation       |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan                    |
| CG | Congo                    | KR | Republic of Korea                     | SE | Sweden                   |
| CH | Switzerland              | KZ | Kazakhstan                            | SI | Slovenia                 |
| CI | Côte d'Ivoire            | LI | Liechtenstein                         | SK | Slovakia                 |
| CM | Cameroon                 | LK | Sri Lanka                             | SN | Senegal                  |
| CN | China                    | LU | Luxembourg                            | TD | Chad                     |
| CS | Czechoslovakia           | LV | Latvia                                | TG | Togo                     |
| CZ | Czech Republic           | MC | Monaco                                | TJ | Tajikistan               |
| DE | Germany                  | MD | Republic of Moldova                   | TT | Trinidad and Tobago      |
| DK | Denmark                  | MG | Madagascar                            | UA | Ukraine                  |
| ES | Spain                    | ML | Mali                                  | US | United States of America |
| FI | Finland                  | MN | Mongolia                              | UZ | Uzbekistan               |
| FR | France                   |    |                                       | VN | Viet Nam                 |
| GA | Gabon                    |    |                                       |    |                          |

-1-

**HUMAN MUTATOR GENE hMSH2 AND HEREDITARY NON  
POLYPOSIS COLORECTAL CANCER**

This invention was made using U.S. government grants from the NIH CA47527, CA09320, GM26449, CA09243, CA41183, CA42705 CA57435, and CA35494, as well as grants from the Department of Energy DOE/ERN/F139 and DE-FG 09291ER-61139. Therefore the U.S. government retains certain rights to the invention.

This application is a continuation-in-part of Serial No. 08/056,546, filed May 5, 1993.

**TECHNICAL FIELD OF THE INVENTION**

The invention relates to a gene which predisposes individuals to colorectal and other cancers. In addition, it also relates to biochemical tests which can be used to identify drugs for treatment of affected individuals.

**BACKGROUND OF THE INVENTION**

HNPCC (Lynch syndrome) is one of the most common cancer predisposition syndromes, affecting as many as 1 in 200 individuals in the western world (Lynch et al., 1993). Affected individuals develop tumors of the colon, endometrium, ovary and other organs, often before 50 years of age. Although the familial nature of this syndrome was discovered nearly a century ago (Warthin et al., 1913), the role of heredity in its causation remained difficult to define (Lynch et al., 1966). Recently, however, linkage analysis in two large kindreds demonstrated association with polymorphic markers on chromosome 2 (Peltomaki

SUBSTITUTE SHEET (RULE 26)

- 2 -

et al., 1993a). Studies in other families suggested that neoplasia in a major fraction of HNPCC kindreds is linked to this same chromosome 2p locus (Aaltonen et al., 1993).

HNPCC is defined clinically by the occurrence of early-onset colon and other specific cancers in first degree relatives spanning at least two generations (Lynch et al., 1993). The predisposition is inherited in an autosomal dominant fashion. It was initially expected that the gene(s) responsible for HNPCC was a tumor suppressor gene, as other previously characterized cancer predisposition syndromes with this mode of inheritance are caused by suppressor gene mutations (reviewed in Knudson, 1993). But the analysis of tumors from HNPCC patients suggested a different mechanism. Most loci encoding tumor suppressor genes undergo somatic losses during tumorigenesis (Stanbridge, 1990). In contrast, both alleles of chromosome 2p loci were found to be retained in HNPCC tumors (Aaltonen et al., 1993). During this search for chromosome 2 losses, however, it was noted that HNPCC tumors exhibited somatic alterations of numerous microsatellite sequences.

Widespread, subtle alterations of the cancer cell genome were first detected in a subset of sporadic colorectal tumors using the arbitrarily-primed polymerase chain reaction (Peinado et al., 1992). These alterations were subsequently found to represent deletions of up to 4 nucleotides in genomic polyA tracts (Ionov et al., 1993). Other studies showed that a similar, distinctive subgroup of sporadic tumors had insertions or deletions in a variety of simple repeated sequences, particularly microsatellite sequences consisting of dinucleotide or trinucleotide repeats (Ionov et al., 1993; Thibodeau et al., 1993; Aaltonen et al., 1993). Interestingly, these sporadic tumors had certain features in common with those developing in HNPCC kindreds, such as a tendency to be located on the right side of the colon and to be near-diploid. These and other data suggested that HNPCC and a subset of sporadic tumors were associated with a heritable defect causing replication errors (RER) of microsatellites (Ionov et al., 1993; Aaltonen et al., 1993).

SUBSTITUTE SHEET (RULE 26)

- 3 -

The mechanism underlying the postulated defect could not be determined from the study of tumor DNA, but studies in simpler organisms provided an intriguing possibility (Levinson and Gutman, 1987; Strand et al., 1993). This work showed that bacteria and yeast containing defective mismatch repair genes manifest instability of dinucleotide repeats. The disruption of genes primarily involved in DNA replication or recombination had no apparent effect on the fidelity of microsatellite replication (reviewed in Kunkel, 1993). These pivotal studies suggested that defective mismatch repair might be responsible for the microsatellite alterations in the tumors from HNPCC patients (Strand et al., 1993).

Thus there is a need in the art to identify the actual gene and protein responsible for hereditary non-polyposis colorectal cancer and the replication error phenotype found in both hereditary and sporadic tumors. Identification of the gene and protein would allow more widespread diagnostic screening for hereditary non-polyposis colorectal cancer than is currently possible. Identification of the involved gene and protein would also enable the rational screening of compounds for use in drug therapy of hereditary non-polyposis colorectal cancer, and would enable gene therapy for affected individuals.

#### **SUMMARY OF THE INVENTION**

It is an object of the invention to provide a DNA molecule which when mutated is the genetic determinant for hereditary non-polyposis colorectal cancer.

It is another object of the invention to provide DNA molecules which contain specific mutations which cause hereditary non-polyposis colorectal cancer.

It is yet another object of the invention to provide methods of treating persons who are predisposed to hereditary non-polyposis colorectal cancer.

It is still another object of the invention to provide methods for determining a predisposition to cancer.

It is a further object of the invention to provide methods for screening test compounds to identify therapeutic agents for treating persons predisposed to hereditary non-polyposis colorectal cancer.

SUBSTITUTE SHEET (RULE 26)

It is still another object of the invention to provide a protein which is important for human DNA mismatch repair.

It is yet another object of the invention to provide a transgenic animal for studying potential therapies for hereditary non-polyposis colorectal cancer.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment of the invention an isolated and purified DNA molecule is provided. The molecule has a sequence of at least about 20 nucleotides of *hMSH2*, as shown in SEQ ID NO:1.

In another embodiment of the invention an isolated and purified DNA molecule is provided. The DNA molecule has a sequence of at least about 20 nucleotides of an *hMSH2* allele found in a tumor wherein said DNA molecule contains a mutation relative to *hMSH2* shown in SEQ ID NO:1.

In yet another embodiment of the invention a method of treating a person predisposed to hereditary non-polyposis colorectal cancer is provided. The method prevents accumulation of somatic mutations. The method involves administering a DNA molecule which has a sequence of at least about 20 nucleotides of *hMSH2*, as shown in SEQ ID NO:1, to a person having a mutation in an *hMSH2* allele which predisposes the person to hereditary non-polyposis colorectal cancer, wherein said DNA molecule is sufficient to remedy the mutation in an *hMSH2* allele of the person.

In another embodiment of the invention a method is provided for determining a predisposition to cancer. The method involves testing a body sample of a human to ascertain the presence of a mutation in *hMSH2* which affects *hMSH2* expression or *hMSH2* protein function, the presence of such a mutation indicating a predisposition to cancer.

In still another embodiment of the invention a method is provided for screening to identify therapeutic agents which can prevent or ameliorate tumors. The screening method involves contacting a test compound with a purified *hMSH2* protein or a cell; determining the ability of the *hMSH2* protein or the cell to perform DNA mismatch repair, a test compound which increases the ability of said

- 5 -

*hMSH2* protein or said cell to perform DNA mismatch repair being a potential therapeutic agent.

In another embodiment of the invention an isolated and purified protein is provided. The protein has the sequence shown in SEQ ID NO:2.

In still another embodiment of the invention a transgenic animal is provided. The transgenic (nonhuman) animal maintains an *hMSH2* allele in its germline. The *hMSH2* allele is one which is found in humans having hereditary non-polyposis colorectal cancer or in RER<sup>+</sup> tumors. Also provided are animals which have no wild-type *MSH2* alleles, due to mutations introduced.

Thus the present invention provides the art with the sequence of the gene responsible for hereditary non-polyposis colorectal cancer and information regarding the mechanism by which it causes tumors. This enables the art to practice a variety of techniques to identify persons at risk of developing a variety of cancers and to treat them to prevent such cancers from actually developing.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 summarizes the markers retained in somatic cell hybrids used in locating the *hMSH2* gene.

PCR was used to determine whether each of the listed markers was present (black box) or absent (white box) in the indicated hybrid. The laboratory name of each hybrid and the formal name (in parentheses) is listed. The hybrid panel was also validated with ten additional polymorphic markers outside of the 136-177 region. M: hybrid derived from microcell-mediated chromosome 2 transfer; T: derived from t(X;2) translocation; X: derived from X-irradiated chromosome 2 donor. MO: mouse-human hybrid; HA: hamster-human hybrid; RA: rat-human hybrid; TEL: telomere; CEN: centromere.

Figure 2 shows a FISH analysis which was used to determine the proximity and ordering of DNA sequences within chromosome band 2p16.

Panels 2A and 2B show FISH mapping of the 123 marker. Panel 2A shows G-banded metaphase chromosome 2. Panel 2B shows identical chromosome as in

SUBSTITUTE SHEET (RULE 26)

- 6 -

Panel 2A following FISH with a biotin-labeled P1 clone for the 123 marker. Results localize the 123 marker to chromosome band 2p16.3. Panels 2C and 2D show co-hybridization documenting the coincident localization of a microdissection (Micro-FISH) probe from chromosome 2p16 and the 123 marker. Panel 2C shows DAPI stained metaphase chromosome 2. Panel 2D shows simultaneous hybridization of the biotin-labeled 123 probe (appearing as an intensely staining smaller circle) and the Spectrum-Orange labeled 2p16 Micro-FISH probe (appearing as a diffusely staining larger circle). Panel 2E shows a representative example of an interphase nucleus simultaneously hybridized with P1 clones for CA5, *hMSH2* and *ze3*. The results were used to directly measure the distances between markers in order to establish the order and relative distance between markers (after Trask et al., 1989). Inset: The image processing program NIH *Image* was used to provide an average gray value displayed as a surface plot to support the length measurements and to graphically illustrate the relative order information. The surface plot presented defines the specified interphase chromosome and the relative order CA-*MSH2*-*ze3*.

Figure 3 shows linkage analysis of HNPCC pedigrees.

All affected individuals in which meiotic recombination occurred between markers 119 and 136 are included. A black box indicates that the individual did not contain the allele associated with disease in his/her family or that the individual inherited an allele not associated with disease from his/her affected parent. A white box indicates that the individual had an allele which was the same size as the disease-associated allele. A hatched circle indicates that the marker was not studied. All individuals had colon or endometrial cancer at less than 55 years of age, or had progeny with such disease but did not indicate that the patient necessarily had disease-associated alleles because phase could usually not be determined.

Figure 4 shows *hMSH2* gene localization.

Southern blots containing EcoRI (figures 4A and 4C) and PstI (figures 4B and 4D) digested DNA from the indicated somatic cell hybrids (figures 4A and 4B)

SUBSTITUTE SHEET (RULE 26)



- 7 -

or YAC clones (figures 4C and 4D) were hybridized with a radiolabelled insert from cDNA clone pNP-23. Southern blotting and hybridization were performed as described (Vogelstein et al., 1987). Autoradiographs are shown. The 5.0 kb PstI fragment in hybrids Z11 and Z12 is derived from hamster DNA.

Figure 5 shows the cDNA sequence of *hMSH2*.

An open reading frame (ORF) begins at nucleotide 1 and ends at nt 2802. The predicted amino acid sequence is shown. The sequence downstream of nt 2879 was not determined.

Figure 6 shows homology between yeast and human *MSH2* genes.

The predicted amino acid sequences of yeast (y) *MSH2* (Reenan and Kolodner, 1992) and human *MSH2* genes are compared within the region of highest homology. Blocks of similar amino acids are shaded.

Figure 7 shows germline and somatic mutations of *hMSH2*.

Autoradiographs of polyacrylamide gels containing the sequencing reactions derived from PCR products are shown. The 1.4 kb PCR products containing a conserved region of *hMSH2* were generated from genomic DNA samples as described in the Examples. Antisense primers were used in the sequencing reactions. The ddA mixes from each sequencing reaction were loaded in adjacent lanes to facilitate comparison, as were those for C, G, and T. The DNA samples were derived from the tumor (lane 1) and normal colon (lane 2) of patient Cx10, an RER- colon tumor cell line (lane 3), and lymphocytes of patients J-42 (lane 4) and J-143 (lane 5). Figure 7A: A transition (C to T at codon 622) in lymphocyte DNA can be observed in HNPCC patients J-42 and J-143. Figure 7B: A transition (C to T at nt codon 639) in tumor (lane 1) and normal colonic mucosa (lane 2) of patient Cx10. Figure 7C: A substitution of a TG dinucleotide for an A at codon 663 can be observed in DNA of the tumor of patient Cx10, (lane 1), but not in DNA from her normal colon (lane 2). Arrows mark the substitutions in panel A and B and the TG dinucleotide insertion site in panel C.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

The disclosure of Serial No. 08/056,546, filed May 5, 1993, is expressly incorporated herein.

It is a discovery of the present invention that the gene responsible for hereditary non-polyposis colorectal cancer is *hMSH2*, a human analog of bacterial *MutS*. The cDNA sequence of *hMSH2* is shown in SEQ ID NO:1. This gene encodes a DNA mismatch repair enzyme. Mutation of the gene causes cells to accumulate mutations. For example, the observed replication error phenotype (RER<sup>+</sup>) found in both sporadic and hereditary non-polyposis colorectal cancer consists of variations (insertions and deletions) in microsatellite DNA. In yeast and bacteria defective *MutS*-related genes cause other types of mutations as well.

Useful DNA molecules according to the invention are those which will specifically hybridize to *hMSH2* sequences. Typically these are at least about 20 nucleotides in length and have the nucleotide sequence as shown in SEQ ID NO:1. Such molecules can be labeled, according to any technique known in the art, such as with radiolabels, fluorescent labels, enzymatic labels, sequence tags, etc. According to another aspect of the invention, the DNA molecules contain a mutation which has been found in tumors of HNPCC patients or in sporadic RER<sup>+</sup> tumors. Such molecules can be used as allele-specific oligonucleotide probes to track a particular mutation through a family.

According to some aspects of the invention, it is desirable that the DNA encode all or a part of the *hMSH2* protein as shown in SEQ ID NO:2. To obtain expression of the protein the DNA sequence can be operably linked to appropriate control sequences, such as promotor, Kozak consensus, and terminator sequences.

A person who is predisposed to develop cancers due to inheritance of a mutant *hMSH2* allele can be treated by administration of a DNA molecule which contains all or a part of the normal *hMSH2* gene sequence as shown in SEQ ID NO:1. A portion of the gene sequence will be useful when it spans the location of the mutation which is present in the mutant allele, so that a double recombination event between the mutant allele and the normal portion "corrects"

- 9 -

the defect present in the person. A portion of the gene can also be usefully administered when it encodes enough of the protein to express a functional DNA mismatch repair enzyme. Such a portion need not necessarily recombine with the mutant allele, but can be maintained at a separate locus in the genome or on an independently replicating vector. Means for administering DNA to humans are known in the art, and any can be used as is convenient. A variety of vectors are also known for this purpose. According to some techniques vectors are not required. Such techniques are well known to those of skill in the art.

Also contemplated as part of the present invention is the use of a combined anti-neoplastic therapy regimen. Such a combined regimen is useful for patients having an RER<sup>+</sup> tumor, whether sporadic or associated with HNPCC. The regimen combines any standard anti-neoplastic therapy to which a patient can become resistant and *hMSH2* gene therapy, as described above. By remedying the defect present in RER<sup>+</sup> cells, *i.e.*, an *hMSH2* mutation, the likelihood of the tumor developing a resistance mutation is greatly diminished. By delaying or preventing the onset of resistance, the life of cancer patients can be prolonged. In addition, such prevention of resistance allows a greater degree of tumor destruction by the therapeutic agent. Examples of anti-neoplastic therapies which can be combined with *hMSH2* gene therapy are hormones, radiation, cytotoxic drugs, cytotoxins, and antibodies.

Body samples can be tested to determine whether the *hMSH2* gene is normal or mutant. Mutations are those deviations from the sequence shown in SEQ ID NO:1 which are associated with disease and which cause a change in *hMSH2* protein function or expression. Such mutations include nonconservative amino acid substitutions, deletions, premature terminations and frameshifts. See Table I. Suitable body samples for testing include those comprising DNA, RNA, or protein, obtained from biopsies, blood, prenatal, or embryonic tissues, for example.

Provided with the information that the defect causing HNPCC and sporadic RER<sup>+</sup> tumors is in a DNA mismatch repair enzyme, one can perform assays on

SUBSTITUTE SHEET (RULE 26)

- 10 -

test compounds and compositions to determine if they will remedy the defect. Such therapeutic compounds could bind to missense *hMSH* mutant proteins to restore the proteins to the normal, active conformation. Alternatively such therapeutic compounds could stimulate the expression of alternate pathways for mismatch repair. Screening for such therapeutic compounds could be performed by contacting test compounds with cells, either normal or those with an *hMSH2* mutation found in a tumor. The ability of the cells which were contacted with the test compounds is compared with the ability of the same cells which were not contacted for mismatch repair activity. Such activity can be tested as is known in the art. See, for example, Levinson and Gutman, 1987, and Strand et al., 1993. Observation of changes in microsatellite DNA in cells is one way of assessing mismatch repair activity. Another approach is to assay DNA mismatch repair *in vitro* in nuclear extracts. See Holmes, 1990; Thomas, 1991; and Fang, 1993.

SUBSTITUTE SHEET (RULE 26)

- 11 -

Table I

| Sample   | Source        | Type     | Codon   | cDNA<br>Nucleotide Change | Predicted<br>Coding Change |
|----------|---------------|----------|---------|---------------------------|----------------------------|
| Family J | HNPCC Kindred | Germline | 622     | CCA to CTA                | Pro to Leu                 |
| Family C | HNPCC Kindred | Germline | 265-314 | 793 to 942 Deletion       | Inframe Deletion           |
| Family 8 | HNPCC Kindred | Germline | 406     | CGA to TGA                | Arg to Stop                |
| Cx10     | RER+ Tumor    | Germline | 639     | CAT to TAT                | His to Tyr                 |
|          |               | Somatic  | 663     | ATG to TG'IG              | Frameshift                 |

SUBSTITUTE SHEET (RULE 26)

- 12 -

Provided with the cDNA sequence and the amino acid of *hMSH2* protein, one of ordinary skill in the art can readily produce *hMSH2* protein, isolated and purified from other human proteins. For example, recombinant cells or organisms can be used to produce the protein in bacteria, yeast, or other convenient cell system. The isolated and purified protein can be used in screening for new therapeutic agents, for example, in *in vitro* assays of DNA mismatch repair. The protein can also be used to raise antibodies against *hMSH2*. Therapeutic administration of the protein is also contemplated.

Transgenic animals are also contemplated by the present invention. These animals would have inserted in their germline *hMSH2* alleles which are associated with HNPCC or sporadic tumors. Such animals could provide model systems for testing drugs and other therapeutic agents to prevent or retard the development of tumors. Also contemplated are genetically engineered animals which contain one or more mutations in their own *MSH2* genes. The mutations will be engineered to correspond to mutations found in *hMSH2* alleles which are found in HNPCC-affected individuals or in other human RER<sup>+</sup> tumors. Animals with both native *MSH2* alleles inactivated and containing a human wild-type or mutant *hMSH2* allele are particularly desirable.

SUBSTITUTE SHEET (RULE 26)

## **Examples**

### **Example 1**

#### **Somatic Cell Hybrids**

A panel of human-hamster, human-mouse, and human-rat hybrid cell lines was developed to facilitate HNPCC mapping. Hybrids containing only portions of chromosome 2 were obtained by microcell-mediated chromosome transfer or by standard cell fusions following X-irradiation of the chromosome 2 donor. Additionally, two hybrids were used which contained a (X;2)(q28;p21) translocation derived from human fibroblasts. In previous studies, the HNPCC locus was mapped to the 25 cM region surrounding marker 123 and bordered by markers 119 and 136 (Peltomaki et al., 1993a). Thirty-eight hybrids were screened with these three chromosome 2p markers. Eight of the hybrids proved useful for mapping the relevant portion of chromosome 2p. For example, hybrids L1 and L2 contained the distal half of the region, including marker 123, while hybrid y3 contained the half proximal to marker 123 (Figure 1).

#### **Methods**

Methods for the derivation of microcell-mediated chromosome 2 hybrids have been described previously (Chen et al., 1992; Spurr et al., 1993). Some hybrids were generated following fusion of X-irradiated donor cells containing human chromosome 2 to CHO cells (Chen et al., 1994). Mouse hybrids were derived by fusing HPRT deficient L cells (A9) with human fibroblasts (GM7503) containing a t(X;2)(q28,p21) translocation and selecting in media containing HAT.

### **Example 2**

#### **Polymorphic Markers**

To map more finely the HNPCC locus, additional polymorphic markers were obtained in three ways. First, a genomic clone containing 85 kb surrounding the 123 marker was used for fluorescence *in situ* hybridization (FISH) to localize it to chromosomal band 2p16.3 (Figure 2A,B). The 2p16 band region was then microdissected, and the sequences within this band were amplified using the polymerase chain reaction and subcloned into plasmid vectors (see Experimental

- 14 -

Procedures). The accuracy of the microdissection was confirmed using dual-color FISH by simultaneously hybridizing to microdissected material and a genomic clone containing marker 123 (Figure 2C,D). The subclones were screened by hybridization to a (CA)<sub>15</sub> probe, and hybridizing clones identified and sequenced. These sequences were then used to design oligonucleotide primers for PCR analysis of genomic DNA. Nineteen (CA)<sub>n</sub> repeat markers were identified in this way. Of these, four were highly polymorphic and mapped to the region between markers 119 and 136, as assessed by the somatic cell hybrid panel exhibited in Figure 1. Second, eight additional (CA)<sub>n</sub> markers, cloned randomly from human genomic DNA using a poly (CA) probe, were found to lie between markers 119 and 136 by linkage analysis in CEPH pedigrees. Five of these were particularly informative and were used in our subsequent studies. Finally, one additional marker was identified by screening subclones of a genomic P1 clone containing marker 123 with a (CA)<sub>15</sub> probe. Through these analyses, thirteen new polymorphic markers were identified in the 25 cM interval between markers 119 and 136, resulting in an average marker spacing of ~2 cM (Table II). These markers were mapped with respect to one another by linkage in CEPH and HNPCC pedigrees as well as by analysis of somatic cell hybrids. These two mapping techniques provided consistent and complementary information. For example, the relative positions of CA16 and CA18 could not be distinguished through linkage analysis but could be determined with the somatic cell hybrids L1, L2, and Y3. Conversely, the relative position of the ze3 and yh5 markers could not be determined through somatic cell hybrid mapping, but could be discerned by linkage analysis.

SUBSTITUTE SHEET (RULE 26)



TABLE II

| MARKER            | DERIVATION | CM  | LOD  | HETEROZYGOSITY | YAC<br>CLONES       | P1<br>CLONES |
|-------------------|------------|-----|------|----------------|---------------------|--------------|
| 177 (AFM267zc9)   | T          | --- |      | 0.84           |                     |              |
| 119 (AFM077yb7)   | T          | 6.1 | 5.5  | 0.77           | 11E1                | 408          |
| yh5 (AFM337yh5)   | T          | 6.4 | 15.4 | 0.76           | 4F4, 1E1            | 838,839, 840 |
| ze3 (AFM200ze3)   | T          | 0.0 | ---  | 0.61           | 4F4, 1E1, 9H8, 4A10 | 838,837      |
| CA5 (CA5)         | M          | 2.1 | 4.7  | 0.77           | 7F10, 4E2, 5A11     | 820          |
| CA7 (CA7)         | M          | 1.7 | 3    | 0.78           | 688                 |              |
| 123 (AFM093xh3)   | T          | 2.4 | 9.9  | 0.76           | 3D11, 8C7           | 210, 211     |
| CA2 (CA2)         | P          | 0.0 | ---  | 0.75           | 3D11, 8C7           | 210, 211     |
| CA18 (CA18)       | M          | 4.3 | 17.1 | 0.71           | 8E5                 |              |
| CA18 (CA18)       | M          | 0.0 | ---  | 0.69           | 8E5                 |              |
| yb9 (AFM320yb9)   | T          | 1.1 | 3.9  | 0.80           | 284                 |              |
| x15 (AFM310x15)   | T          | 2.7 | 17.6 | 0.76           |                     |              |
| x11 (AFM348i11)   | T          | 0.0 | ---  | 0.79           |                     |              |
| 147 (AFM199vb6)   | T          | 2.6 | 9.8  | 0.73           |                     |              |
| 136 (AFM172xe7)   | T          | 2   |      | 0.73           |                     |              |
| 134 (AFM 168xg11) | T          | 1   |      | 0.76           |                     | 387,388, 389 |

SUBSTITUTE SHEET (RULE 26)

## Methods

All markers were obtained by screening human genomic libraries with radiolabelled (CA)<sub>n</sub> probes (Weber and May, 1989). The "T" markers (see Table 1) were generated from a library made from total human genomic DNA, as described in Weissenbach et al., 1992. The "M" markers were made from libraries generated from microdissected chromosome 2p16, as described below. The CA2 marker was generated from a library made from P1 clone 210 digested to completion with Sau3 and cloned into the XhoI site of lambda YES (Elledge et al., 1991). The sequences of the clones obtained from these libraries were determined, and primers surrounding the CA repeats chosen. Only primers giving robust amplification and high heterozygosity were used for detailed analysis of HNPCC kindreds. All markers used in this study were shown to be derived from chromosome 2p by both linkage analysis in the CEPH pedigrees and evaluation in the somatic cell hybrid panel shown in Figure 1. The sequences of the primers and other details specific for each marker have been deposited with the Genome Data Base. Linkage analyses to obtain the map of the marker loci in CEPH families 1331, 1332, 1347, 1362, 1413, 1416, 884, and 102 (Weissenbach et al., 1992) were performed using the CLINK program of the LINKAGE program package (Lathrop et al., 1984) with the no sex difference option and 11-point computations. The odds for the best locus order supported by the data were evaluated against pairwise inversions of the loci.

## Example 3

### Genomic Clones

Many of the polymorphic markers shown in Table 1 were used to derive genomic clones containing 2p16 sequences. Genomic clones were obtained by PCR screening of human P1 and YAC libraries with these polymorphic markers, with ten additional sequence tagged sites (STS) derived from chromosome 2p16 microdissection, or with YAC junctions. Twenty-three P1 clones, each containing 85-95 kb, were obtained, as well as 35 YAC clones, containing 300 to 1800 kb. The YAC clones in some cases confirmed the linkage and somatic cell hybrid

- 17 -

maps. For example, markers ze3 and yh5 were both found in YAC's 4F4 and 1E1 while CA16 and CA18 were both found in YAC 8E5, documenting their proximity. The highest density of genomic clones (28 YAC and 17 P1 clones) was obtained between markers yb9 and yh5 (Table 1), which became the region most likely to contain the HNPCC gene during the course of these studies. The region between yh5 and yb9 was predicted to contain ~ 9 Mb (assuming 1 Mb/per cM). Based on the sizes of the YAC clones, and taking into account their chimerism, we estimated that they contained over 70% of the sequences between yh5 and yb9.

#### **Methods**

The markers described in Table 1 were used to screen YAC or P1 libraries by PCR. The CEPH A library was obtained from Research Genetics, Inc. and consisted of 21,000 YAC clones, arrayed in a format allowing facile screening and unambiguous identification of positive clones. The sizes of ten of the YAC clones containing markers were determined by transverse alternating pulse-field gel electrophoresis using a GeneLine II apparatus from Beckman and found to average 0.7 Mb (range 0.2-1.8 Mb). In some cases, inverse PCR was used to determine the YAC junctions (Joslyn et al., 1991), and the derived sequence used for "chromosome walking" with the YAC or P1 libraries. The junctions were also used to design primers to test whether the ends of the YAC clones could be localized to chromosome 2p16 (and therefore presumably be non-chimeric). Three of four YAC clones which were tested in this way had both ends within the expected region of chromosome 2, as judged by analysis with the somatic cell hybrid panel. The human genomic P1 library was also screened by PCR (Genome Systems, Inc.). P1 clones M1015 and M1016, containing the *hMSH2* gene, were used to determine intron-exon borders using sequencing primers from the exons and SequiTherm™ polymerase (Epicentre Technologies).

#### **Example 4**

##### **Analysis of HNPCC Families**

The markers described in Table 1 were then used to analyze six large HNPCC kindreds previously linked to chromosome 2p (Peltomaki et al., 1993a).

SUBSTITUTE SHEET (RULE 26)

Two hundred thirteen individuals, including 56 members affected with colorectal or endometrial cancer, were examined. Four of the kindreds were from the United States, one from Newfoundland and one from New Zealand. To increase the number of affected individuals that could be examined, we obtained formalin-fixed, paraffin-embedded sections of normal tissues from deceased individuals and purified DNA from them (Goelz et al., 1985a). A single allele of each of the thirteen markers was found to segregate with disease in each of the six families (i.e., the allele was found in over 50% of affected individuals). No allele of any marker was shared among the affected members of more than three kindreds.

Fourteen of the affected members contained only a subset of the expected alleles and therefore had undergone recombination between markers 119 and 136. Eleven of these individuals appeared to have simple, single recombination events. The most informative of these was in individual 148 from the J kindred and individual 44 from kindred 621 (Figure 3). Individual 621-44 apparently retained the disease linked allele at markers distal to CA5, while demonstrating multiple recombinants at more proximal loci, thus placing the CA5 marker at the proximal border of the HNPCC locus. Individual J-148 apparently retained the disease-linked allele at all markers proximal to yh5, while exhibiting recombinants at yh5 and 119, thus placing the distal border at yh5. Assuming that the same gene was involved in both the J and 621 kindreds, the HNPCC gene was predicted to reside between markers CA5 and yh5, an area spanning approximately 2 cM (Table 1).

The DNA of three affected individuals (C-202, 4-156, 4-92) appeared to have undergone two recombinations in the area. There was probably one recombinant per generation, and this could be demonstrated in C-202 by analysis of DNA from his parents; in the other cases, parental DNA was not available. All three individuals retained disease-linked alleles at CA5 and ze3 but not at more proximal and distal loci (Figure 3). Combined with the data from the patients with single recombinations, the double recombinants suggested that the HNPCC gene resided between CA5 and ze3, a distance spanning less than 2 cM.

SUBSTITUTE SHEET (RULE 26)

- 19 -

To determine the physical distances separating CA5, ze3, and yh5, metaphase and interphase FISH analysis was carried out. Dual-color FISH with P1 clones containing these markers was performed with P1 clones 820 and 838 (containing the markers CA5 and yh5, respectively) labeled with biotin and detected with fluorescein-labelled avidin, and clone 836 (containing marker ze3) labelled with Spectrum-Orange (Meltzer et al., 1992). The hybridization signals of these markers appeared coincident on metaphase chromosomes, confirming that they resided within an interval of <1.0 Mb. When FISH was performed on interphase nuclei, the relative positions of the three markers could be determined and the distances between them estimated (Trask et al., 1989). The results confirmed that the orientation of the markers was telomere-yh5-ze3-CA5-centromere (data not shown). Direct measurement of the distance between yh5 and ze3 was estimated at <0.3 Mb, consistent with the presence of both of these markers on YAC clones 4E4 and 1E1. Measurements of 48 interphase chromosomes provided an estimate of the distance between ze3 and CA5 at <0.8 Mb, independently confirming the linkage data.

#### Methods

G-banded metaphase chromosomes were microdissected with glass microneedles and amplified by PCR as previously described (Guan et al., 1993; Kao and Yu, 1991). For dual-color FISH, the PCR product was fluorochrome labelled (Spectrum-Orange, Imagenetics, Naperville, IL) or biotinylated in a secondary PCR reaction. P1 clones were labelled by nick-translation or by degenerate oligonucleotide primers (Guan et al., 1993). FISH was carried out as previously described (Guan et al., 1993) and visualized with a Zeiss Axiophot equipped with a dual-bandpass filter. For analysis of interphase FISH patterns, the distance between hybridization signals was measured in a minimum of 24 nuclei (Trask et al., 1989).

SUBSTITUTE SHEET (RULE 26)

### Example 5

#### Candidate Genes

On the basis of the mapping results described above, we could determine whether a given gene was a candidate for HNPCC by determining its position relative to the CA5-ze3 domain. The first gene considered was a human homolog of the *Drosophila* *SOS* gene (reviewed by Egan and Weinberg, 1993). This gene transmits signals from membrane bound receptors to the ras pathway in diverse eukaryotes. It was considered a candidate because another ras-interacting gene, *NF1*, causes a cancer predisposition syndrome (Viskochil et al., 1990; Wallace et al., 1990), and *SOS* has been localized to chromosome 2p16-21 by *in situ* hybridization (Webb et al., 1993). Using PCR to amplify *SOS* sequences from the hybrid panel, however, *SOS* was found to be distal to the CA5-yh5 domain (present in hybrid Z19 but not Z29).

We next examined the interferon-inducible RNA activated protein kinase gene *PKR*. This gene has been shown to have tumor suppressor ability (reviewed by Lengyel, 1993) and to map close to 2p16 (Hanash et al., 1993). We could not initially exclude *PKR* from the HNPCC domain, and therefore determined the sequence of its coding region in two individuals from HNPCC kindred C. Reverse transcriptase was used to generate cDNA from lymphoblastoid derived RNA of these two individuals, and PCR performed with primers specific for *PKR*. The *PKR* products were sequenced, and no deviations from the published sequence was identified within the coding region (Meurs et al., 1990). Subsequent studies showed that the *PKR* gene was distal to the yh5 marker, and thus could be excluded as a basis for HNPCC.

We then considered human homologs of the MutL and MutS mismatch repair genes previously shown to produce microsatellite instability in bacteria and yeast when disrupted (Levinson and Gutman, 1987; Strand et al., 1993). A human homolog of the yeast *MutL*-related gene *PMS1* (Kramer et al., 1989) does not appear to reside on chromosome 2p (M. Liskay, personal communication). To identify homologs of *MutS*, we used degenerate oligonucleotide primers to PCR-

- 21 -

amplify cDNA from colon cancer cell lines. The same primers had been previously used to identify the yeast *MSH2* gene on the basis of its *MutS* homology (Reenan and Kolodner, 1992). Under non-stringent conditions of PCR, a fragment of the expected size was obtained and these fragments were cloned into plasmid vectors. Most of the clones contained ribosomal RNA genes, representing abundant transcripts with weak homology to the degenerate primers. A subset of the clones, however, contained sequences similar to that of the yeast *MSH2* gene, and one such clone, pNP-23, was evaluated further. The human gene from which this clone is derived is hereafter referred to as *hMSH2*.

The insert from clone pNP-23 was used as a probe in Southern blots of somatic cell hybrid DNA. This insert hybridized to one or two fragments in human genomic DNA digested with *Pst*I or *Eco*RI, respectively, and these fragments were present in hybrid Z30, containing most of human chromosome 2p. Analysis of other hybrids showed that the fragment was present in hybrids Z11, Z29, L1 and L2, but not Z12, Y3 or Z19, thereby localizing the human *MSH2* (*hMSH2*) gene to a region bordered by markers CA18 and 119 (examples in Figure 4). The YAC clones listed in Table 1 were then analyzed, and *Eco*RI and *Pst*I fragments of the expected size identified in YAC 5A11, derived from screening the YAC library with the CA5 marker (Figure 4).

To confirm the Southern blots, we designed non-degenerate primers on the basis of the sequence of pNP-23. Several sets of primers were tested so that genomic DNA could be used as a template for PCR; an intervening intron prevented the original primers from being used effectively with templates other than cDNA. PCR with these primers was perfectly consistent with the Southern blot results. The expected 101 bp fragment was present in hybrids Z4, Z11, Z29, L1, and L2, and in YAC 5A11, but not in other hybrids or YAC clones (not shown).

The localization of *hMSH2* sequences to YAC 5A11 demonstrated the proximity of these sequences to marker CA5. To determine the distance and relative orientation of *hMSH2* with respect to CA5, we performed interphase FISH

SUBSTITUTE SHEET (RULE 26)

analysis. P1 clones containing CA5, ze3 and *hMSH2* sequences (clones 820, 836, and M1015, respectively) were simultaneously hybridized to interphase nuclei following fluorescein and Spectrum Orange labelling (Meltzer et al., 1992). The results demonstrated that *MSH2* resides within the HNPCC locus defined by linkage analysis to lie between CA5 and ze3, and less than 0.3 Mb from marker CA5 (Figure 2E).

cDNA libraries generated from human colon cancer cells or from human fetal brain tissues were then screened with the insert of pNP-23 to obtain additional sequences from this gene. Seventy-five cDNA clones were initially identified and partially sequenced. PCR products representing the ends of the cDNA sequence contig were then used as probes to re-screen the cDNA libraries. This cDNA "walk" was repeated again with the new contig ends. Altogether, 147 cDNA clones were identified. The composite sequence derived from these clones is shown in Figure 5. An open reading frame (ORF) began 69 nt downstream of the 5' end of the cDNA contig, and continued for 2802 bp. The methionine initiating this ORF was in a sequence context compatible with efficient translation (Kozak, 1986) and was preceded by in-frame termination codons. RNA from placenta and brain were used in a PCR-based procedure (RACE, Frohman et al., 1988) to independently determine the position of the 5' end of *hMSH2* transcripts. This analysis demonstrated that the 5' ends of all detectable transcripts were less than 100 bp upstream of the sequence shown in Figure 5, and were heterogeneous upstream of nt -69. The region of highest homology to the yeast *MSH2* gene is shown in Figure 6. This region encompassed the helix-turn-helix domain perhaps responsible for MutS binding to DNA (Reenan and Kolodner, 1992). The yeast and human *MSH2* proteins were 77% identical between codons 615 and 788. There were several other blocks of similar amino acids distributed throughout the length of these two proteins (966 and 934 amino acids in yeast and human, respectively).

#### Methods



- 23 -

cDNA generated from the RNA of colorectal cancer cells with reverse transcriptase was used as template for PCR with the degenerate primers 5'-CTG GAT CCA C(G/A/T/C) G G(G/A/T/C)C C(G/A/T/C)A A(T/C)A TG-3' and 5'-CTG GAT CC(G/A) TA(G/A) TG(G/A/T/C) GT (G/A/T/C) (G/A)C(G/A) AA-3'. These two primers were used previously to identify the yeast *MSH2* gene and were based on sequences conserved among related mammalian and bacterial genes (Reenan and Kolodner, 1992). The optimal PCR conditions for detecting the human *MSH2* gene consisted of 35 cycles at 95° for 30 seconds, 41° for 90 seconds, and 70° for 90 seconds, in the buffer described previously (Sidransky et al., 1991). PCR products were cloned into T-tailed vectors as described (Holton and Graham, 1991) and sequenced with modified T7 polymerase (USB). The insert from one clone (pNP-23) containing human sequences homologous to the yeast *MSH2* gene was then used to screen cDNA libraries generated from RNA of SW480 colon cancer cells (Clontech) or of fetal brain (Stratagene). After two further rounds of screening, positive clones were converted into plasmids and sequenced using modified T7 polymerase (Kinzler et al., 1991). In some cases, the inserts were amplified using one *hMSH2*-specific primer and one vector-specific primer, and then sequenced with SequiTherm Polymerase (Epicentre Technologies). To determine the 5' end of *MSH2* transcripts, RACE was performed (Frohman et al., 1989) using brain and placenta cDNA (Clontech).

#### Example 6

##### Mutations of *hMSH2*

The physical mapping of *hMSH2* to the HNPCC locus was intriguing but could not prove that this gene was responsible for the disease. To obtain more compelling evidence, we determined whether germ line mutations of *hMSH2* were present in the two HNPCC kindreds that originally established linkage to chromosome 2 (Peltomaki et al., 1993a). Intron-exon borders within the most conserved region of *hMSH2* (Figure 6) were determined by sequencing genomic PCR fragments containing adjacent exons. Genomic DNA samples from the lymphocytes of affected members of these two kindreds were then used as

SUBSTITUTE SHEET (RULE 26)

- 24 -

templates for PCR to determine the sequence of this domain. The DNA from individual J-42, afflicted with colon and endometrial cancer at ages 42 and 44, respectively, was found to contain one allele with a C to T transition at codon 622 (CCA to CTA), resulting in a substitution of leucine for proline (Figure 7, top). Twenty additional DNA samples from unrelated individuals all encoded proline at this position. Twenty one members of the J kindred were then analyzed by direct sequencing of PCR products. All eleven affected individuals contained one allele with a C to T transition in codon 622, while all ten unaffected members contained two normal alleles, thus documenting perfect segregation with disease. Importantly, this proline was at a highly conserved position, the identical residue being found in all known MutS related genes from prokaryotes and eukaryotes (Figure 6 and Reenan and Kolodner, 1992).

No mutations of the conserved region of *MSH2* were identified in kindred C, so we next examined other parts of the *hMSH2* transcript. RNA was purified from lymphoblastoid cells of patient C-202, a 27 year old male with colon cancer. Reverse transcriptase coupled PCR (RT-PCR) was used to generate four *hMSH2*-specific products encompassing codons 89 to 934 from this RNA (see Experimental Procedures). An abnormal, smaller RT-PCR product was identified with one of the primer pairs used. Mapping and sequencing studies using various *MSH2* primers showed that the abnormal product was the result of a presumptive splicing defect which removed codons 265 to 314 from the *hMSH2* transcript. The abnormal transcript was found to segregate with disease in the C kindred, and was not found in twenty unrelated individuals.

We next wished to determine whether *hMSH2* was altered in one of the more recently linked families (R.P. and M.N-L., unpublished data), and chose kindred 8 for detailed analysis. DNA and RNA were obtained from lymphoblastoid cells of 8-143, a 42 year old male with colon cancer. The conserved region of *hMSH2* was amplified from genomic DNA using PCR and directly sequenced. A T to C substitution was noted in the polypyrimidine tract upstream of the exon beginning at codon 669 (at intron position -6). However,

SUBSTITUTE SHEET (RULE 26)

this substitution was also found in two of twenty unrelated, normal individuals, and was therefore a polymorphism unrelated to the disease, with an allele frequency of 0.05. Most of the *hMSH2* coding region was then amplified by RT-PCR, as described above, and no abnormal transcripts were detected. Sequencing of the PCR products, however, revealed a C to T transition at codon 406 (CGA to TGA) causing substitution of a termination codon for an arginine residue. RNA was available from the lymphocytes of a second affected member of kindred 8, and the same stop codon was identified. This alteration was not found in twenty other, unrelated individuals.

Finally, we wished to determine whether mutations of this gene occurred in RER<sup>+</sup> tumors from patients without evident family histories of cancer. The conserved region of *MSH2* was studied in four colorectal tumor cell lines from such patients using genomic DNA as templates for PCR. One tumor (from patient Cx10) was found to contain two *hMSH2* alterations. The first was a C to T transition in codon 639 (CAT to TAT), resulting in a substitution of tyrosine for histidine. This change was not found in any of twenty samples from unrelated individuals, but was present in the DNA from normal colon of this patient, and was therefore likely to represent a germ line change (Figure 7, middle). Like the missense mutation in the J kindred, the Cx10 alteration was at a position perfectly conserved in all MutS homologs (Figure 6 and Reenan and Kolodner, 1992). The second alteration in the tumor from Cx10 was a substitution of a GT dinucleotide for an A in codon 663 (ATG to TGTG). The resultant one bp insertion was predicted to cause a frameshift, producing a termination codon 36 nt downstream. This mutation was demonstrated in both RNA and DNA purified from the Cx10 tumor, but was not present in the patient's normal colon, so represented a somatic mutation (Figure 7, bottom). The PCR products from Cx10 were cloned and sequenced, and the insertion mutation at codon 663 and the transition at codon 639 were shown to reside on different alleles.

- 26 -

### Methods

To detect mutations, PCR products were generated from cDNA and human genomic DNA templates, then sequenced directly using SequiTherm™. In some cases, the PCR products were cloned into T-tailed vectors for sequencing to confirm the direct sequencing data. The primers used to amplify the conserved region of *MSH2* from genomic DNA were 5'-CCA CAA TGG ACA CTT CTG C-3' and 5'-CAC CTG TTC CAT ATG TAC G-3', resulting in a 1.4 kb fragment containing *hMSH2* codons 614 to 705, and primers 5'-AAA ATG GGT TGC AAA CAT GC-3' and 5'-GTG ATA GTA CTC ATG GCC C-3', resulting in a 2.0 kb fragment containing *MSH2* cDNA codons 683 to 783. Primers for RT-PCR were 5'-AGA TCT TCT TCT GGT TCG TC-3' and 5'-GCC AAC AAT AAT TTC TGG TG-3' for codons 89 to 433, 5'-TGG ATA AGA ACA GAA TAG AGG-3' and 5'-CCA CAA TGG ACA CTT CTG C-3' for codons 350-705, 5'-CAC CTG TTC CAT ATG TAC G-3' and 5'-AAA ATG GGT TGC AAA CAT GC-3' for codons 614 to 783, and 5'-GTG ATA GTA CTC ATG GCC C-3' and 5'-GAC AAT AGC TTA TCA ATA TTA CC-3' for codons 683-949.

### Discussion

Three major conclusions can be drawn from the examples described here. First, physical mapping and linkage analysis localized the HNPCC locus on chromosome 2 to an 0.8 Mb segment bordered by markers CA5 and ze3. Second, a new human homolog of the yeast *MSH2* gene was identified, and this gene shown to lie in the same 0.8 Mb interval. Third, alterations of the *hMSH2* gene occurred in the germ line of patients with RER<sup>+</sup> tumors, with or without classical HNPCC, and additional somatic alterations of this gene occurred in tumors (Summarized in Table I). The alterations were at highly conserved regions or significantly altered the expected gene product and thus represent mutations with important functional effects. These results indicate that mutations of *hMSH2* are responsible for HNPCC and the RER<sup>+</sup> positive phenotype found in tumors.

SUBSTITUTE SHEET (RULE 26)

- 27 -

These data have substantial implications for understanding the neoplastic disease observed in HNPCC. In particular, they suggest that the microsatellite alterations previously observed in tumors from these patients are not epiphenomena, but are intrinsically related to pathogenesis. Additionally, the mutations observed in yeast and bacteria with defective MutS-related genes are not confined to insertions and deletions at simple repeated sequences, though these sequences provide convenient tools for analysis (Modrich, 1991). Similarly, one would expect that many mutations, in addition to microsatellite insertions or deletions, would be found in HNPCC tumors. This could lead to the multiple, sequential mutations in oncogenes and tumor suppressor genes which have been shown to drive colorectal tumorigenesis (Fearon and Vogelstein, 1990). Thus, the molecular pathogenesis of HNPCC tumors is likely to be similar to that occurring in non-HNPCC cases, though accelerated by the increased rate of mutation associated with mismatch repair defects. Accordingly, colon tumors from HNPCC patients have been shown to contain mutations of APC, p53 and RAS at frequencies similar to those found in sporadic colorectal cancers (Aaltonen et al., 1993).

Colorectal tumors from HNPCC patients are distinguished by their relatively normal cytogenetic composition (Kouri et al., 1990), and sporadic, RER<sup>+</sup> tumors have been demonstrated to have substantially fewer chromosome losses than those occurring in RER<sup>-</sup> cases (Thibodeau et al., 1993; Aaltonen et al., 1993). These data suggest that genetic heterogeneity is critical for colorectal cancer development, but can be generated in two different ways (Thibodeau et al., 1993). Most commonly, it develops through gross alterations resulting in aneuploidy, as suggested nearly eighty years ago (Boveri, 1914). In HNPCC-derived tumors and RER<sup>+</sup> sporadic tumors, the diversity is presumably more subtle, consisting of multiple small sequence changes distributed throughout the genome. The latter mechanism of generating diversity may be less dangerous to the host, as HNPCC patients, as well as patients with RER<sup>+</sup> sporadic tumors, appear to have a better prognosis than would be expected from histopathologic

SUBSTITUTE SHEET (RULE 26)

analysis of their tumors (Ionov et al., 1993; Thibodeau et al., 1993; Lothe et al., 1993; Lynch et al., 1993).

### References

Aaltonen, L.A., Peltomaki, P., Leach, F.S., Sistonen, P., Pylkkanen, L., Mecklin, J-P., Jarvinen, H., Powell, S.M., Jen, J., Hamilton, S.R., Petersen, G.M., Kinzler, K.W., Vogelstein, B., and de la Chapelle, A. (1993). Clues to the pathogenesis of familial colorectal cancer. *Science* 260, 812-816.

Baylin, S.B., Makos, M., Wu, J., C-Y, R-W., de Bustros, A., Vertino, P., and Nelkin, B.D. (1991). Abnormal patterns of DNA methylation in human neoplasia: Potential consequences for tumor progression. *Cancer Cells* 3, 383, 390.

Boveri, T. (1914). *Zur Frage der Entstehung maligner tumoren*. Gustave Fischer Verlag, Jena, Vol. 1.

Chen, D.J., Park, M.S., Campbell, E., Oshimura, M., Liu, P., Zhao, Y., White, B.F., and Siciliano, M.J. (1992). Assignment of a human DNA double-strand break repair gene (XRCC5) to chromosome 2. *Genomics* 13, 1088-1094.

Chen, D.J., Marrone, B., Nguyen, T., Stackhouse, M., Zhao, Y., and Sicilano, J.J., (1994). Regional assignment of a human radiation repair gene (XRCC5) to 2q35 by X-ray hybrid mapping. *Genomics*, in press.

Egan, S.E., and Weinberg, R.A. (1993). The pathway to signal achievement. *Nature* 365, 781-783.

Elledge, S.J., Mulligan, J.T., Ramer, S.W., Spottswood, M., and Davis R.W. (1991). Lambda YES: a multifunctional cDNA expression vector for the isolation

- of genes by complementation of yeast and *Escherichia coli* mutations. Proc. Natl. Acad. Sci. USA 88, 1731-1735.
- Fang, W.-h. and P. Modrich (1993). Human strand-specific mismatch repair occurs by a bidirectional mechanism similar to that of the bacterial reaction. J. Biol. Chem. 268, 11838-11844.
- Fearon, E.R., and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. Cell 61, 759-767.
- Frohman, M.A., Dush, M.K., and Martin, G.R. (1988). Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA 85, 8998-9002.
- Goelz, S.E., Hamilton, S.R., and Vogelstein, B. Purification of DNA from Formaldehyde Fixed and Paraffin Embedded Human Tissue. (1985a). Biochem. Biophys. Res. Commun. 130, 118-126.
- Goelz, S.E., Vogelstein, B., Hamilton, S.R., and Feinberg, A.P. Hypomethylation of DNA from Benign and Malignant Human Colon Neoplasms. (1985b). Science 228, 187-190.
- Guan, X.-Y., Trent, J.M., and Meltzer, P.S. (1993). Generation of band-specific painting probes from a single microdissected chromosome. Human Molecular Genetics 2, 1117-1121.
- Han, H-J., Yanagisawa, A., Kato, Y., Park, J-G., and Nakamura, Y. (1993). Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Research 53, 5087-5089.

Hanash, S.M., Beretta, L., Barcroft, C.L., Sheldon, S., Glover, T.W., Ungar, D., and Sonenberg, N. (1993). Mapping of the gene for interferon-inducible dsRNA-dependent protein kinase to chromosome region 2p21-22: A site of rearrangements in myeloproliferative disorders. *Genes, Chromosomes & Cancer* 8, 34-37.

Holmes, J., S. Clark and P. Modrich (1990). Strand-specific mismatch correction in nuclear extracts of human and *Drosophila melanogaster* cell lines. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5837-5841.

Holton, T.A., and Graham, M.W. (1991). A simple and efficient method for direct cloning of PCR products using ddT-tailed vectors. *Nucleic Acids Research* 19, 1156.

Ionov, Y.M., Peinado, A., Malkhosyan, S., Shibata, D., and Perucho M. (1993). Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 363, 558-561.

Jass, J.R., Stewart, S.M. (1992). Evolution of hereditary non-polyposis colorectal cancer. *Gut* 33, 783-786.

Joslyn, G., Carlson, M., Thilveris, A., Albertsen, H., Gelbert, L., Samowitz, W., Groden, J., Stevens, J., Spirio, L., Robertson, M., Sargeant, L., Krapcho, K., Wolff, E., Burt, R., Hughes, J.P., Warrington, J., McPherson, J., Wasmuth, J., Le Paslier, D., Abderrahim, H., Cohen, D., Leppert, M., and White, R. (1991). Identification of deletion mutations and three new genes at the familial polyposis locus. *Cell* 66, 601-613.



- 31 -

Kao, F-T., and Yu, J-W. (1991). Chromosome microdissection and cloning in human genome and genetic disease analysis. *Proc. Natl. Acad. Sci. USA* 88, 1844-1848.

Kinzler, K.W., Nilbert, M.C., Su, L.-K., Vogelstein, B., Bryan, T.M., Levy, D.B., Smith, K.J., Preisinger, A.C., Hedge, P., McKechnie, D., Finniear, R., Markham, A., Groffen, J., Boguski, M.S., Altschul, S.F., Horii, A., Ando, H., Miyoshi, Y., Miki, Y., Nishisho, I., Nakamura, Y. Identification of FAP Locus Genes from Chromosome 5q21. (1991). *Science* 253, 661-665.

Kouri, M., Laasonen, A., Mecklin, J.P., Jarvinen, H., Franssila, K., Pyrhonen, S. (1990). Diploid predominance in hereditary nonpolyposis colorectal carcinoma evaluated by flow cytometry. *Cancer* 65, 1825-1829.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by Eukaryotic Ribosomes. *Cell* 44, 283-292.

Kramer, W., Kramer, B., Williamson, M.S., Fogel, S. (1989). Cloning and nucleotide sequence of DNA mismatch repair gene PMS1 from *Saccharomyces cerevisiae*: homology of PMS1 to procaryotic MutL and HexB. *Journal of Bacteriology* 171, 5339-5346.

Knudson, A.G. (1993). All in the (cancer) family. *Nature Genetics* 5, 103-104.

Kunkel, T.A. (1993). Slippery DNA and diseases. *Nature* 365, 207-208.

Lathrop, G.M., Lalouel, J.M., Julier, C., and Ott, J. (1984). Strategies for multilocus linkage analysis in humans. *Proc. Natl. Acad. Sci. USA* 81, 3443-3446.

SUBSTITUTE SHEET (RULE 26)

Lengyel, P. (1993). Tumor-suppressor genes: News about the interferon connection. Proc. Natl. Acad. Sci. USA 90, 5893-5895.

Levinson, G., and Gutman, G.A. (1987). High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. Nucleic Acids Research 15, 5323-5338.

Lindblom, A., Tannergard, P., Werelius, B., and Nordenskjold, M. (1993). Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. Nature Genetics 5, 279-282.

Lothe, R.A., Peltomaki, P., Meling, G-I., Aaltonen, L.A., Nystrom-Lahti, M., Pylkkanen, L., Heimdal, K., Andersen, T.I., Moller, P., Rognum, T.O., Fossa, S.D., Haldorsen, T., Langmark, F., Bragger, A., de la Chapelle, A., and Barresea, A-L. (1993). Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. Cancer Research, in press.

Lynch, H.T., Omaha, M.W., Shaw, M.D. (1966). Hereditary factors in cancer. Arch Intern Med 117, 206-212.

Lynch, H.T., Smyrk, T.C., Watson, P., Lanspa, S.J., Lynch, J.F., Lynch, P.M., Cavalieri, R.J., and Boland, C.R. (1993). Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: An updated review. Gastroenterology 104, 1535-1549.

Meltzer, P.S., Guan, X-Y., Burgess, A., and Trent, J.M. (1992). Rapid generation of region specific probes by chromosome microdissection and their application. Nature Genetics 1, 24-28.

Meurs, E., Chong, K., Galabru, J., Thomas, N.S.B., Kerr, I.M., Williams, B.R.G., and Hovanessian, A.G. (1990). Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. *Cell* 62, 379-390.

Modrich, P. (1991). Mechanisms and biological effects of mismatch repair. *Ann. Rev. Genet.* 25, 229-253.

Peinado, M.A., Malkhosyan, S., Velazquez, A., and Perucho, M. (1992). Isolation and characterization of allelic losses and gains in colorectal tumors by arbitrarily primed polymerase chain reaction. *Proc. Natl. Acad. Sci. USA* 89, 10065-10069.

Peltomaki, P., Aaltonen, L.A., Sistonen, P., Pylkkanen, L., Mecklin, J-P., Jarvinen, H., Green, J.S., Jass, J.R., Weber, J.L., Leach, F.S., Petersen, G.M., Hamilton, S.R., de la Chapelle, A., and Vogelstein, B. (1993a). Genetic mapping of a locus predisposing to human colorectal cancer. *Science* 260, 810-812.

Pukkila, P.J., Petseon, J., Herman, G., Modrich, P., and Meselson, M. (1983). Effects of high levels of DNA adenine methylation on methyl-directed mismatch repair in *Escherichia coli*. *Genetics* 104, 571-582.

Reenan, R.A., Kolodner, R.D. (1992). Isolation and characterization of two *Saccharomyces cerevisiae* genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins. *Genetics* 132, 963-973.

Risinger, J.I., Berchuck, A., Kohler, M.F., Watson, P., Lynch, H.T., and Boyd, J. (1993). Genetic instability of microsatellites in endometrial carcinoma. *Cancer Research* 53, 5100-5103.

SUBSTITUTE SHEET (RULE 26)

Sidransky, D., von Eschenbach, A., Tsai, Y.C., Jones, P., Summerhayes, I., Marshall, F., Paul, M., Green, P., Hamilton, S.R., Frost, P., Vogelstein, B. (1991). Identification of p53 Gene Mutations in Bladder Cancers and Urine Samples. *Science* 252, 706-709.

Spurr, N.K., Cox, S., Naylor, S. (1993). Report and abstracts of the Second International Workshop on human chromosome 2 mapping. *Cytogenetics & Cell Genetics* 64, 69-92.

Stanbridge, E.J. (1990). Human tumor suppressor genes. *Ann. Rev. Genes* 24, 615-657.

Strand, M., Prolla, T.A., Liskay, R.M., and Petes, T.D. (1993). Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* 365, 274-276.

Thibodeau, S.N., Bren, G., and Schaid, D. (1993). Microsatellite instability in cancer of the proximal colon. *Science* 260, 816-819.

Thomas, D.C., J.D. Roberts and T.A. Kunkel (1991). Heteroduplex repair in extracts of human HeLa cells. *J. Biol. Chem.* 266, 3744-3751.

Trask, B., Pinkel, D., and van den Engh, G. (1989). The proximity of DNA sequences in interphase cell nuclei is correlated to genomic distance and permits ordering of cosmids spanning 250 kilbase pairs. *Genomics* 5, 710-717.

Viskochil, D., Buchberg, A.M., Xu, G., Cawthon, R., Stevens, J., Wolff, R.K., Culver, M., Carey, J.C., Copeland, N.G., Jenkins, N.A., White, R., and O'Connell, P. (1990). Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell* 62, 187-192.

- 35 -

Vogelstein, B., Fearon, E.R., Hamilton, S.R., Preisinger, A.C., Willard, H.F., Michelson, A.M., Riggs, A.D., and Orkin, S.H. (1987). Clonal Analysis Using Recombinant DNA Probes from the X Chromosome. *Cancer Research* 47, 4806-4813.

Wallace, M.R., Marchuk, D.A., Andersen, L.B., Letcher, R., Odeh, H.M., Saulino, A.M., Fountain, J.W., Brereton, A., Nicholson, J., Mitchell, A.L., Brownstein, B.H., Collins, F.S. (1990). Type 1 neurofibromatosis gene: Identification of a large transcript disrupted in three NF1 patients. *Science* 249, 181-186.

Warthin, A.S. (1913). Heredity with reference to carcinoma: As shown by the study of the cases examined in the pathological laboratory of the University of Michigan, 1895-1913. *Arch. Int. Med* 12, 546-555.

Watson, P., and Lynch, H.T. (1993). Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer* 71, 677-685.

Webb, G.C., Jenkins, N.A., Largaespada, D.A., Copeland, N.G., Fernandez, C.S., and Bowtell, D.D.L. (1993). Mammalian homologues of the *Drosophila* *son of sevenless* gene map to murine chromosomes 17 and 12 and to human chromosomes 2 and 14, respectively. *Genomics* 18, 14-19.

Weber, J.L., May P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* 44, 338-396.

Weissenbach, J., Gyapay, G., Dib, C., Vignal A., Morissette, J., Millasseau, P., Vaysseix, G., and Lathrop, M. (1992). A second-generation linkage map of the human genome. *Nature* 359, 794-801.

SUBSTITUTE SHEET (RULE 26)

- 36 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Vogelstein, Bert  
Kinzler, Kenneth W.  
de la Chappelle, Albert
- (ii) TITLE OF INVENTION: Mutator Gene and Hereditary  
Non-Polyposis Colorectal Cancer
- (iii) NUMBER OF SEQUENCES: 16
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Banner, Birch, McKie, and Beckett
  - (B) STREET: 1001 G Street, N.W.
  - (C) CITY: Washington
  - (D) STATE: D.C.
  - (E) COUNTRY: USA
  - (F) ZIP: 20001
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/160295
  - (B) FILING DATE: 02-DEC-1993
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Kagan, Sarah A.
  - (B) REGISTRATION NUMBER: 32,141
  - (C) REFERENCE/DOCKET NUMBER: 01107.44900
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 202.508.9100
  - (B) TELEFAX: 202.508.9299
  - (C) TELEX: 197430 BBMB UT

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2947 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

SUBSTITUTE SHEET (RULE 26)

- 37 -

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

|   |      |
|---|------|
| GGCGGGAAAC AGCTTAGTGG GTGTGGGGTC GCGCATTTTC TTCAACCAGG AGGTGAGGAG   | 60   |
| GTTTCGACAT GGCGGTGCAG COGAAGGAGA CGCTGCAGTT GGAGAGCGCG GCCGAGGTCTG  | 120  |
| GCTTCGTGCG CTTCTTTTCAG GGCATGCCGG AGAAGCCGAC CACCACAGTG CGCCTTTTTCG | 180  |
| ACCGGGGCGA CTTCTATACG GCGCACGGCG AGGACGCGCT GCTGGCCGCC CGGGAGGTGT   | 240  |
| TCAAGACCCA GGGGGTGATC AAGTACATGG GGCCGGCAGG AGCAAAGAAT CTGCAGAGTG   | 300  |
| TTGTGCTTAG TAAAATGAAT TTTGAATCTT TTGTAAAAGA TCTTCTTCTG GTTCGTCACT   | 360  |
| ATAGAGTTGA AGTTTATAAG AATAGAGCTG GAAATAAGGC ATCCAAGGAG AATGATTGGT   | 420  |
| ATTTGGCATA TAAGGCTTCT CCTGGCAATC TCTCTCAGTT TGAAGACATT CTCTTTGGTA   | 480  |
| ACAATGATAT GTCAGCTTCC ATTGGTGTTG TGGGTGTTAA AATGTCCGCA GTTGATGGCC   | 540  |
| AGAGACAGGT TGGAGTTGGG TATGTGGATT CCATACAGAG GAAACTAGGA CTGTGTGAAT   | 600  |
| TCCCTGATAA TGATCAGTTC TCCAATCTTG AGGCTCTCCT CATCCAGATT GGACCAAAGG   | 660  |
| AATGTGTTTT ACCCGGAGGA GAGACTGCTG GAGACATGGG GAAACTGAGA CAGATAATTC   | 720  |
| AAAGAGGAGG AATTCTGATC ACAGAAAGAA AAAAGCTGA CTTTTCCACA AAAGACATTT    | 780  |
| ATCAGGACCT CAACCGGTTG TTGAAAGGCA AAAAGGGAGA GCAGATGAAT AGTGCTGTAT   | 840  |
| TGCCAGAAAT GGAGAATCAG GTTGCAGTTT CATCACTGTC TCGGTAATC AAGTTTTTAG    | 900  |
| AACTCTTATC AGATGATTCC AACTTTGGAC AGTTTGAAC TACTACTTTT GACTTCAGCC    | 960  |
| AGTATATGAA ATTGGATATT GCAGCAGTCA GAGCCCTTAA CCTTTTTCAG GGTTCTGTTG   | 1020 |
| AAGATACCAC TGGCTCTCAG TCTCTGGCTG CCTTGCTGAA TAAGTGTAAG ACCCCTCAAG   | 1080 |
| GACAAAGACT TGTTAACCAG TGGATTAAGC AGCCTCTCAT GGATAAGAAC AGAATAGAGG   | 1140 |
| AGAGATTGAA TTTAGTGGAA GCTTTTGTAG AAGATGCAGA ATTGAGGCAG ACTTTACAAG   | 1200 |
| AAGATTTACT TCGTCGATTC CCAGATCTTA ACCGACTTGC CAAGAAGTTT CAAAGACAAG   | 1260 |
| CAGCAAACCTT ACAAGATTGT TACCGACTCT ATCAGGGTAT AAATCAACTA CCTAATGTTA  | 1320 |
| TACAGGCTCT GGAAAAACAT GAAGGAAAAC ACCAGAAATT ATTGTTGGCA GTTTTTGTGA   | 1380 |
| CTCCTCTTAC TGATCTTCGT TCTGACTTCT CCAAGTTTCA GGAAATGATA GAAACAACTT   | 1440 |
| TAGATATGGA TCAGGTGGAA AACCATGAAT TCCTTGTAAG ACCTTCATTT GATCCTAATC   | 1500 |
| TCAGTGAATT AAGAGAAATA ATGAATGACT TGGAAAAGAA GATGCAGTCA ACATTAATAA   | 1560 |
| GTGCAGCCAG AGATCTTGGC TTGGACCCTG GCAAACAGAT TAACTGGAT TCCAGTGCAC    | 1620 |
| AGTTTGGATA TTACTTTTCGT GTAACCTGTA AGGAAGAAAA AGTCCTTCGT AACAAATAAA  | 1680 |
| ACTTTAGTAC TGTAGATATC CAGAAGAATG GTGTTAAATT TACCAACAGC AAATTGACTT   | 1740 |
| CTTTAAATGA AGAGTATACC AAAAATAAAA CAGAATATGA AGAAGCCCAG GATGCCATTG   | 1800 |

SUBSTITUTE SHEET (RULE 26)

- 38 -

```

TTAAAGAAAT TGTCAATATT TCTTCAGGCT ATGTAGAACC AATGCAGACA CTCAATGATG 1860
TGTTAGCTCA GCTAGATGCT GTTGTGAGCT TTGCTCACGT GTCAAATGGA GCACCTGTTT 1920
CATATGTACG ACCAGCCATT TTGGAGAAAG GACAAGGAAG AATTATATTA AAAGCATCCA 1980
GGCATGCTTG TGTGAAGTT CAAGATGAAA TTGCATTTAT TCCTAATGAC GTATACTTTG 2040
AAAAAGATAA ACAGATGTTT CACATCATT CTGGCCCCAA TATGGGAGGT AAATCAACAT 2100
ATATTCGACA AACTGGGGTG ATAGTACTCA TGGCCCAAAT TGGGTGTTTT GTGCCATGTG 2160
AGTCAGCAGA AGTGTCCATT GTGGACTGCA TCTTAGCCCG AGTAGGGGCT GGTGACAGTC 2220
AATTGAAAGG AGTCTCCAGG TTCATGGCTG AAATGTTGGA AACTGCTTCT ATCCTCAGGT 2280
CTGCAACCAA AGATTCATTA ATAATCATAG ATGAATTGGG AAGAGGAACT TCTACCTACG 2340
ATGGATTTGG GTTAGCATGG GCTATATCAG AATACATTGC AACAAAGATT GGTGCTTTTT 2400
GCATGTTTGC AACCATTTTT CATGAACTTA CTGCCTTGGC CAATCAGATA CCAACTGTTA 2460
ATAATCTACA TGTCACAGCA CTCACCACTG AAGAGACCTT AACTATGCTT TATCAGGTGA 2520
AGAAAGGTGT CTGTGATCAA AGTTTTGGGA TTCATGTTGC AGAGCTTGCT AATTTCCCTA 2580
AGCATGTAAT AGAGTGTGCT AAACAGAAAG CCCTGGAAC TGAAGGAGTTT CAGTATATTG 2640
GAGAATCGCA AGGATATGAT ATCATGGAAC CAGCAGCAAA GAAGTGCTAT CTGGAAAGAG 2700
AGCAAGGTGA AAAAATTATT CAGGAGTTCC TGTCCAAGGT GAAACAAATG CCCTTTACTG 2760
AAATGTCAGA AGAAAACATC ACAATAAAGT TAAACACAGT AAAAGCTGAA GTAATAGCAA 2820
AGAATAATAG CTTTGTAAT GAAATCATTT CACGAATAAA AGTTACTACG TGAAAAATCC 2880
CAGTAATGGA ATGAAGGTAA TATTGATAAG CTATTGTCTG TAATAGTTTT ATATTGTTTT 2940
ATATTAA 2947

```

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 934 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: YES

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Val Gln Pro Lys Glu Thr Leu Gln Leu Glu Ser Ala Ala Glu
1           5           10           15

```

SUBSTITUTE SHEET (RULE 26)



- 39 -

Val Gly Phe Val Arg Phe Phe Gln Gly Met Pro Glu Lys Pro Thr Thr  
 20 25 30  
 Thr Val Arg Leu Phe Asp Arg Gly Asp Phe Tyr Thr Ala His Gly Glu  
 35 40 45  
 Asp Ala Leu Leu Ala Ala Arg Glu Val Phe Lys Thr Gln Gly Val Ile  
 50 55 60  
 Lys Tyr Met Gly Pro Ala Gly Ala Lys Asn Leu Gln Ser Val Val Leu  
 65 70 75 80  
 Ser Lys Met Asn Phe Glu Ser Phe Val Lys Asp Leu Leu Leu Val Arg  
 85 90 95  
 Gln Tyr Arg Val Glu Val Tyr Lys Asn Arg Ala Gly Asn Lys Ala Ser  
 100 105 110  
 Lys Glu Asn Asp Trp Tyr Leu Ala Tyr Lys Ala Ser Pro Gly Asn Leu  
 115 120 125  
 Ser Gln Phe Glu Asp Ile Leu Phe Gly Asn Asn Asp Met Ser Ala Ser  
 130 135 140  
 Ile Gly Val Val Gly Val Lys Met Ser Ala Val Asp Gly Gln Arg Gln  
 145 150 155 160  
 Val Gly Val Gly Tyr Val Asp Ser Ile Gln Arg Lys Leu Gly Leu Cys  
 165 170 175  
 Glu Phe Pro Asp Asn Asp Gln Phe Ser Asn Leu Glu Ala Leu Leu Ile  
 180 185 190  
 Gln Ile Gly Pro Lys Glu Cys Val Leu Pro Gly Gly Glu Thr Ala Gly  
 195 200 205  
 Asp Met Gly Lys Leu Arg Gln Ile Ile Gln Arg Gly Gly Ile Leu Ile  
 210 215 220  
 Thr Glu Arg Lys Lys Ala Asp Phe Ser Thr Lys Asp Ile Tyr Gln Asp  
 225 230 235 240  
 Leu Asn Arg Leu Leu Lys Gly Lys Lys Gly Glu Gln Met Asn Ser Ala  
 245 250 255  
 Val Leu Pro Glu Met Glu Asn Gln Val Ala Val Ser Ser Leu Ser Ala  
 260 265 270  
 Val Ile Lys Phe Leu Glu Leu Leu Ser Asp Asp Ser Asn Phe Gly Gln  
 275 280 285  
 Phe Glu Leu Thr Thr Phe Asp Phe Ser Gln Tyr Met Lys Leu Asp Ile  
 290 295 300  
 Ala Ala Val Arg Ala Leu Asn Leu Phe Gln Gly Ser Val Glu Asp Thr  
 305 310 315 320  
 Thr Gly Ser Gln Ser Leu Ala Ala Leu Leu Asn Lys Cys Lys Thr Pro  
 325 330 335  
 Gln Gly Gln Arg Leu Val Asn Gln Trp Ile Lys Gln Pro Leu Met Asp  
 340 345 350

SUBSTITUTE SHEET (RULE 26)

- 40 -

Lys Asn Arg Ile Glu Glu Arg Leu Asn Leu Val Glu Ala Phe Val Glu  
 355 360 365  
 Asp Ala Glu Leu Arg Gln Thr Leu Gln Glu Asp Leu Leu Arg Arg Phe  
 370 375 380  
 Pro Asp Leu Asn Arg Leu Ala Lys Lys Phe Gln Arg Gln Ala Ala Asn  
 385 390 395 400  
 Leu Gln Asp Cys Tyr Arg Leu Tyr Gln Gly Ile Asn Gln Leu Pro Asn  
 405 410 415  
 Val Ile Gln Ala Leu Glu Lys His Glu Gly Lys His Gln Lys Leu Leu  
 420 425 430  
 Leu Ala Val Phe Val Thr Pro Leu Thr Asp Leu Arg Ser Asp Phe Ser  
 435 440 445  
 Lys Phe Gln Glu Met Ile Glu Thr Thr Leu Asp Met Asp Gln Val Glu  
 450 455 460  
 Asn His Glu Phe Leu Val Lys Pro Ser Phe Asp Pro Asn Leu Ser Glu  
 465 470 475 480  
 Leu Arg Glu Ile Met Asn Asp Leu Glu Lys Lys Met Gln Ser Thr Leu  
 485 490 495  
 Ile Ser Ala Ala Arg Asp Leu Gly Leu Asp Pro Gly Lys Gln Ile Lys  
 500 505 510  
 Leu Asp Ser Ser Ala Gln Phe Gly Tyr Tyr Phe Arg Val Thr Cys Lys  
 515 520 525  
 Glu Glu Lys Val Leu Arg Asn Asn Lys Asn Phe Ser Thr Val Asp Ile  
 530 535 540  
 Gln Lys Asn Gly Val Lys Phe Thr Asn Ser Lys Leu Thr Ser Leu Asn  
 545 550 555 560  
 Glu Glu Tyr Thr Lys Asn Lys Thr Glu Tyr Glu Glu Ala Gln Asp Ala  
 565 570 575  
 Ile Val Lys Glu Ile Val Asn Ile Ser Ser Gly Tyr Val Glu Pro Met  
 580 585 590  
 Gln Thr Leu Asn Asp Val Leu Ala Gln Leu Asp Ala Val Val Ser Phe  
 595 600 605  
 Ala His Val Ser Asn Gly Ala Pro Val Pro Tyr Val Arg Pro Ala Ile  
 610 615 620  
 Leu Glu Lys Gly Gln Gly Arg Ile Ile Leu Lys Ala Ser Arg His Ala  
 625 630 635 640  
 Cys Val Glu Val Gln Asp Glu Ile Ala Phe Ile Pro Asn Asp Val Tyr  
 645 650 655  
 Phe Glu Lys Asp Lys Gln Met Phe His Ile Ile Thr Gly Pro Asn Met  
 660 665 670  
 Gly Gly Lys Ser Thr Tyr Ile Arg Gln Thr Gly Val Ile Val Leu Met  
 675 680 685

SUBSTITUTE SHEET (RULE 26)

- 41 -

Ala Gln Ile Gly Cys Phe Val Pro Cys Glu Ser Ala Glu Val Ser Ile  
 690 695 700  
 Val Asp Cys Ile Leu Ala Arg Val Gly Ala Gly Asp Ser Gln Leu Lys  
 705 710 715 720  
 Gly Val Ser Thr Phe Met Ala Glu Met Leu Glu Thr Ala Ser Ile Leu  
 725 730 735  
 Arg Ser Ala Thr Lys Asp Ser Leu Ile Ile Ile Asp Glu Leu Gly Arg  
 740 745 750  
 Gly Thr Ser Thr Tyr Asp Gly Phe Gly Leu Ala Trp Ala Ile Ser Glu  
 755 760 765  
 Tyr Ile Ala Thr Lys Ile Gly Ala Phe Cys Met Phe Ala Thr His Phe  
 770 775 780  
 His Glu Leu Thr Ala Leu Ala Asn Gln Ile Pro Thr Val Asn Asn Leu  
 785 790 795 800  
 His Val Thr Ala Leu Thr Thr Glu Glu Thr Leu Thr Met Leu Tyr Gln  
 805 810 815  
 Val Lys Lys Gly Val Cys Asp Gln Ser Phe Gly Ile His Val Ala Glu  
 820 825 830  
 Leu Ala Asn Phe Pro Lys His Val Ile Glu Cys Ala Lys Gln Lys Ala  
 835 840 845  
 Leu Glu Leu Glu Glu Phe Gln Tyr Ile Gly Glu Ser Gln Gly Tyr Asp  
 850 855 860  
 Ile Met Glu Pro Ala Ala Lys Lys Cys Tyr Leu Glu Arg Glu Gln Gly  
 865 870 875 880  
 Glu Lys Ile Ile Gln Glu Phe Leu Ser Lys Val Lys Gln Met Pro Phe  
 885 890 895  
 Thr Glu Met Ser Glu Glu Asn Ile Thr Ile Lys Leu Lys Gln Leu Lys  
 900 905 910  
 Ala Glu Val Ile Ala Lys Asn Asn Ser Phe Val Asn Glu Ile Ile Ser  
 915 920 925  
 Arg Ile Lys Val Thr Thr  
 930

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

SUBSTITUTE SHEET (RULE 26)

- 42 -

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGGATCCAC NGGNCCNAAY ATG

23

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGGATCCRT ARTGNGTNRC RAA

23

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCACAATGGA CACTTCTGC

19

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACCTGTTCC ATATGTACG

19

SUBSTITUTE SHEET (RULE 26)

- 43 -

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAAATGGGTT GCAAACATGC

20

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTGATAGTAC TCATGGCCC

19

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGATCTTCTT CTGGTTCGTC

20

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:

SUBSTITUTE SHEET (RULE 26)

- 44 -

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCAACAATA ATTTCTGGTG

20

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGGATAAGAA CAGAATAGAG G

21

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCACAATGGA CACTTCTGC

19

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

SUBSTITUTE SHEET (RULE 26)

- 45 -

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CACCTGTTCC ATATGTACG

19

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AAAATGGGTT GCAAACATGC

20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTGATAGTAC TCATGGCCC

19

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

SUBSTITUTE SHEET (RULE 26)

- 46 -

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GACAATAGCT TATCAATATT ACC

23

SUBSTITUTE SHEET (RULE 26)



**CLAIMS**

1. An isolated and purified DNA molecule having a sequence of at least about 20 nucleotides of *hMSH2*, as shown in SEQ ID NO:1.
2. The DNA molecule of claim 1 which is cDNA.
3. The DNA molecule of claim 1 which is labeled.
4. The DNA molecule of claim 1 which is operably linked to a promotor sequence.
5. The DNA molecule of claim 4 which upon transcription produces an RNA molecule having the sequence of native *hMSH2* mRNA.
6. An isolated and purified DNA molecule having a sequence of at least about 20 nucleotides of an *hMSH2* allele found in a tumor wherein said DNA molecule contains a mutation relative to *hMSH2* shown in SEQ ID NO:1.
7. A method of treating a person predisposed to hereditary non-polyposis colorectal cancer to prevent accumulation of somatic mutations, comprising:

administering a DNA molecule which has a sequence of at least about 20 nucleotides of *hMSH2*, as shown in SEQ ID NO:1, to a person having a mutation in an *hMSH2* allele which predisposes the person to hereditary non-polyposis colorectal cancer, wherein said DNA molecule is sufficient to remedy the mutation in an *hMSH2* allele of the person.
8. The method of claim 7 wherein said DNA molecule encodes an *hMSH2* protein as shown in SEQ ID NO:1.
9. The method of claim 7 wherein said DNA molecule spans the mutation site in said allele and remedies the mutation by recombining with said mutant allele.

- 48 -

10. The method of claim 7 wherein said DNA molecule encodes a portion of *hMSH2* protein which is sufficient to provide DNA mismatch repair function.

11. A method of determining a predisposition to cancer comprising:  
testing a body sample of a human to ascertain the presence of a mutation in *hMSH2* which affects *hMSH2* expression or *hMSH2* protein function, the presence of such a mutation indicating a predisposition to cancer.

12. The method of claim 11 wherein the sample is DNA.

13. The method of claim 11 wherein the sample is RNA.

14. The method of claim 11 wherein the sample is protein.

15. The method of claim 11 wherein the sample is isolated from prenatal or embryonic cells.

16. A method of screening to identify therapeutic agents which can prevent or ameliorate tumors, comprising:

contacting a test compound with a cell;

determining the ability of the cell to perform DNA mismatch repair, a test compound which increases the ability of said cell to perform DNA mismatch repair being a potential therapeutic agent.

17. The method of claim 16 wherein the cell contains an *hMSH2* mutation found in a tumor.

18. A method of screening to identify therapeutic agents which can prevent or ameliorate tumors, comprising:

contacting a test compound with an isolated and purified *hMSH2* protein;

determining the ability of the *hMSH2* protein to perform DNA mismatch repair, a test compound which increases the ability of said protein to perform DNA mismatch repair being a potential therapeutic agent.

19. The method of claim 18 wherein the *hMSH2* protein contains a mutation found in a tumor.

SUBSTITUTE SHEET (RULE 26)

- 49 -

20. An isolated and purified protein which has the sequence shown in SEQ ID NO:2.

21. A transgenic animal wherein the transgene is an *hMSH2* allele.

22. The transgenic animal of claim 21 wherein the allele is a mutant allele of *hMSH2* found in a patient having hereditary nonpolyposis colorectal cancer or an RER<sup>+</sup> tumor.

23. A non-human animal which contains no wild-type *MSH2* allele.

24. The transgenic animal of claim 21 wherein said animal contains no wild-type *MSH2* allele.

25. A method of treating a person having an RER<sup>+</sup> tumor to prevent accumulation of somatic mutations leading to resistance to an anti-cancer therapeutic agent, comprising:

administering to a person having a tumor with an RER<sup>+</sup> phenotype: (a) a DNA molecule which has a sequence of at least about 20 nucleotides of *hMSH2*, as shown in SEQ ID NO:1, wherein said DNA molecule is sufficient to remedy a mutation in an *hMSH2* allele of the person; and (b) an anti-neoplastic therapeutic agent.

26. The method of claim 23 wherein the anti-neoplastic therapeutic agent is selected from the group consisting of: a hormone, radiation, a cytotoxic drug, a cytotoxin, and an antibody.

SUBSTITUTE SHEET (RULE 26)

FIG. 1

SOMATIC CELL HYBRIDS

|             | Z4<br>(V15-27) | Z11<br>(6CS5) | Z29<br>(HD(2)11) | Y3<br>F(2n)-21 | Z12<br>(6CS7) | L1<br>(AG3A9) | L2<br>(AG3A10) | Z19<br>(XHB78) |
|-------------|----------------|---------------|------------------|----------------|---------------|---------------|----------------|----------------|
| TEL         |                |               |                  |                |               |               |                |                |
| 177         |                |               |                  |                |               |               |                |                |
| 119         |                |               |                  |                |               |               |                |                |
| yh5         |                |               |                  |                |               |               |                |                |
| ze3         |                |               |                  |                |               |               |                |                |
| CA5         |                |               |                  |                |               |               |                |                |
| CA7         |                |               |                  |                |               |               |                |                |
| 123         |                |               |                  |                |               |               |                |                |
| CA2         |                |               |                  |                |               |               |                |                |
| CA18        |                |               |                  |                |               |               |                |                |
| CA16        |                |               |                  |                |               |               |                |                |
| yb9         |                |               |                  |                |               |               |                |                |
| xf5         |                |               |                  |                |               |               |                |                |
| tf1         |                |               |                  |                |               |               |                |                |
| 147         |                |               |                  |                |               |               |                |                |
| 136         |                |               |                  |                |               |               |                |                |
| CEN         |                |               |                  |                |               |               |                |                |
| DERIVATION: | M              | M             | M                | M              | M             | T             | T              | X              |
| BACKGROUND: | HA             | HA            | HA               | RA             | HA            | MO            | MO             | HA             |

MARKERS

2/10

FIG. 2A



FIG. 2B



FIG. 2C



FIG. 2D

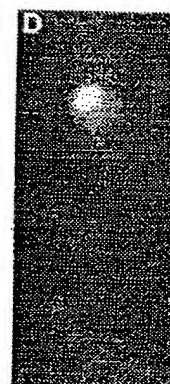
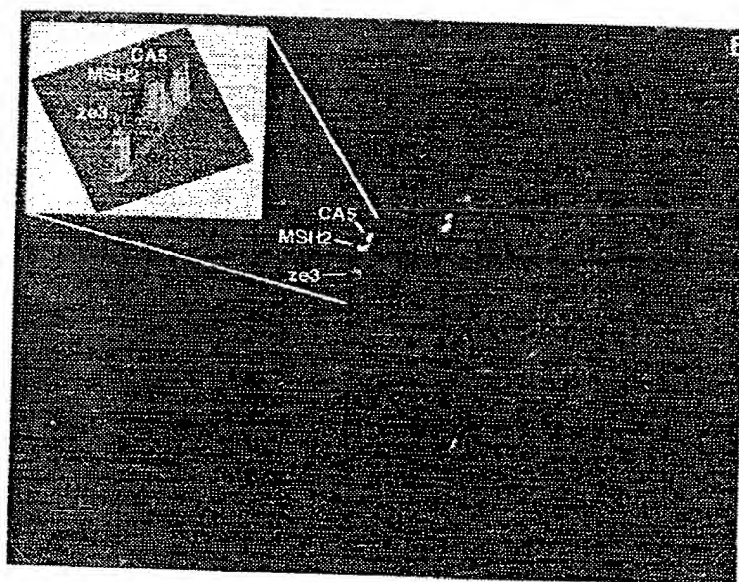


FIG. 2E



SUBSTITUTE SHEET (RULE 26)

3/10

FIG. 3

| KINDRED<br>INDIVIDUAL | C<br>74 | C<br>43 | C<br>202 | J<br>36 | J<br>148 | 4<br>156 | 4<br>92 | 8<br>67 | 8<br>309 | K<br>23 | K<br>37 | 621<br>182 | 621<br>44 | 621<br>119 |
|-----------------------|---------|---------|----------|---------|----------|----------|---------|---------|----------|---------|---------|------------|-----------|------------|
| 119                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| yh5                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| ze3                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| CA5                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| CA7                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| 123                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| CA2                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| CA18                  |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| CA16                  |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| yb9                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| xf5                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| tf1                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| 147                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |
| 136                   |         |         |          |         |          |          |         |         |          |         |         |            |           |            |

SUBSTITUTE SHEET (RULE 26)

4/10

FIG. 4A

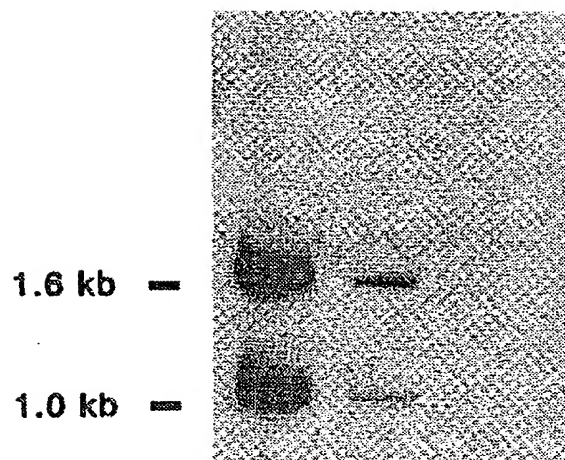
Z  
Z11  
Z12

FIG. 4B

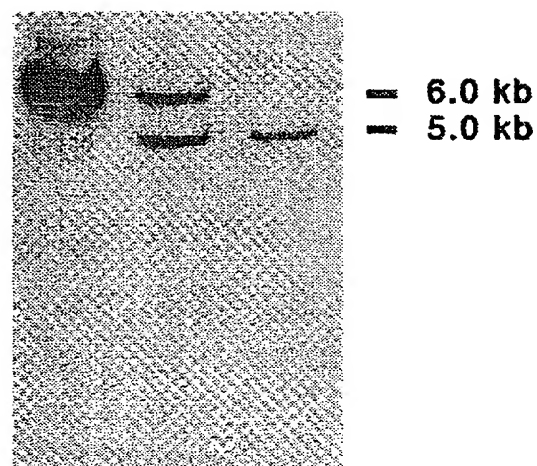
Z  
Z11  
Z12

FIG. 4C

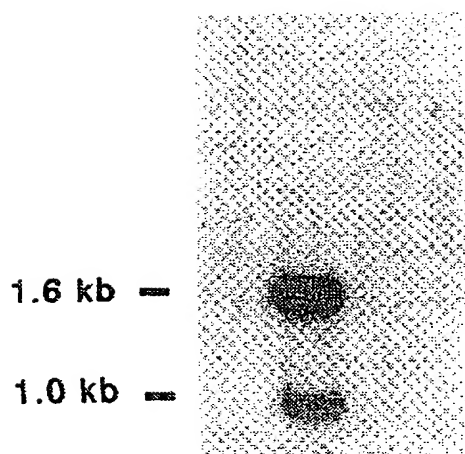
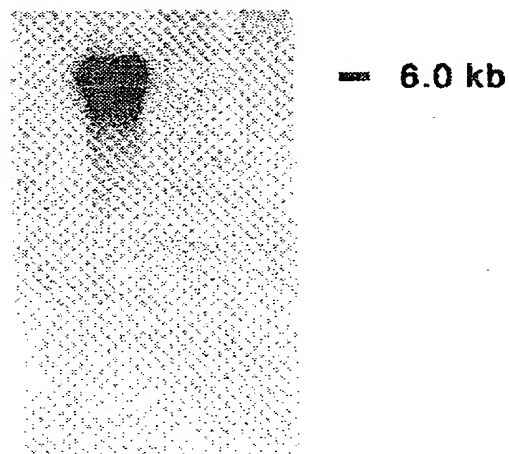
1E1  
5A11  
6B8  
4A10

FIG. 4D

1E1  
5A11  
6B8  
4A10

SUBSTITUTE SHEET (RULE 26)

## FIG. 5A

5/10

Met Ala Val Gln Pro  
 -68 GG CGG GAA ACA GCT TAG TGG GTG TGG GGT CGC GCA TTT TCT TCA ACC AGG TGA GGA GGT TTC GAC ATG GCG GTG CAG CCG  
 6 Lys Glu Thr Leu Gln Leu Glu Ser Ala Ala Glu Val Gly Phe Val Arg Phe Gln Gly Met Pro Glu Lys Pro Thr Thr Thr  
 16 AAG GAG ACG CTG CAG TTG GAG AGC GCG GCC GAG GTC GGC TTC GTG CCC TTC TTT CAG GGC ATG CCG GAG AAG CCG ACC ACC ACA  
 34 Val Arg Leu Phe Asp Arg Gly Asp Phe Tyr Thr Ala His Gly Glu Asp Ala Leu Leu Ala Ala Arg Glu Val Phe Lys Thr Gln  
 100 GTG CGC CTT TTC GAC CCG GCG GAC TTC TAT ACG GCG CAC GCG GAG GAC GCG CTG CTC GCG CCG GAG GTG TTC AAG ACC CAG  
 62 Gly Val Ile Lys Tyr Met Gly Pro Ala Gly Ala Lys Asn Leu Ser Val Val Leu Ser Lys Met Asn Phe Glu Ser Phe Val  
 184 GCG GTG ATC AAG TAC AAG TAC AAG GCG CCG GCA GCA AAG AAT CTG CAG AGT GTT GTG CTT AGT AAA ATG AAT TTT GAA TCT TTT GTA  
 90 Lys Asp Leu Leu Val Arg Gln Tyr Arg Val Glu Val Tyr Lys Asn Arg Ala Gly Asn Lys Ala Ser Lys Glu Asn Asp Trp  
 268 AAA GAT CTT CTT CTG GTT CGT CAG TAT AGA GTT GAA GTT TAT AAG AAT AGA GCT GGA AAT AAG GCA TCC AAG GAG AAT GAT TGG  
 118 Tyr Leu Ala Tyr Lys Ala Ser Pro Gly Asn Leu Ser Gln Phe Glu Asp Ile Leu Phe Gly Asn Asn Asp Met Ser Ala Ser Ile  
 352 TAT TTG GCA TAT AAG GCT TCT CCT GGC AAT CTC TCT CAG TTT GAA GAC ATT CTC TTT GGT AAC AAT GAT ATG TCA GCT TCC ATT  
 146 Gly Val Val Gly Val Lys Met Ser Ala Val Asp Gly Gln Arg Gln Val Gly Val Tyr Val Asp Ser Ile Gln Arg Lys Leu  
 436 GGT GTT GTG GGT GTT AAA ATG TCC GCA GTT GAT GCG CAG AGA CAG GTT GGA GTT GGG TAT GTG GAT TCC ATA CAG AGG AAA CTA  
 174 Gly Leu Cys Glu Phe Pro Asp Asn Asp Gln Phe Ser Asn Leu Glu Ala Leu Leu Ile Gln Ile Gly Pro Lys Glu Cys Val Leu  
 520 GGA CTG TGT GAA TTC CCT GAT AAT GAT CAG TTC TCC AAT CTT GAG GCT CTC CTC ATC CAG ATT GGA CCA AAG GAA TGT GTT TTA  
 202 Pro Gly Gly Glu Thr Ala Gly Asp Met Gly Lys Leu Arg Gln Ile Ile Gln Arg Gly Gly Ile Leu Ile Thr Glu Arg Lys Lys  
 604 CCC GGA GGA GAG ACT GCT GGA GAC ATG GCG AAA CTG AGA CAG ATA ATT CAA AGA GGA GGA ATT CTG ATC ACA GAA AGA AAA AAA  
 230 Ala Asp Phe Ser Thr Lys Asp Ile Tyr Gln Asp Leu Asn Arg Leu Leu Lys Gly Lys Lys Glu Gln Met Asn Ser Ala Val  
 688 GCT GAC TTT TCC ACA AAA GAC ATT TAT CAG GAC CTC AAC CCG TTG TTG AAA GCG AAA AAG GGA GAG CAG ATG AAT AGT GCT GTA  
 258 Leu Pro Glu Met Glu Asn Gln Val Val Ser Ser Leu Ser Ala Val Ile Lys Phe Leu Glu Leu Ser Asp Ser Asn  
 772 TTG CCA GAA ATG GAG AAT CAG GTT GCA GTT TCA TCA CTG TCT GCG GTA ATC AAG TTT TTA GAA CTC TTA TCA GAT GAT TCC AAC  
 286 Phe Gly Gln Phe Glu Leu Thr Thr Phe Asp Phe Ser Gln Tyr Met Lys Leu Asp Ile Ala Ala Val Arg Ala Leu Asn Leu Phe  
 856 TTT GGA CAG TTT GAA CTG ACT ACT TTT GAC TTC AGC CAG TAT ATG AAA TTG GAT ATT GCA GCA GTC AGA GCC CTT AAC CTT TTT

SUBSTITUTE SHEET (RULE 26)



## FIG. 5B

314 Gln Gly Ser Val Glu Asp Thr Thr Gly Ser Gln Ser Leu Ala Ala Leu Leu Asn Lys Cys Lys Thr Pro Gln Gly Gln Arg Leu  
 940 CAG GGT TCT GTT GAA GAT ACC ACT GGC TCT CAG TCT CTG GCT GCC TTG CTG AAT AAG TGT AAA ACC CCT CAA GGA CAA AGA CTT  
  
 342 Val Asn Gln Trp Ile Lys Gln Pro Leu Met Asp Lys Asn Arg Ile Glu Glu Arg Leu Asn Leu Val Glu Ala Phe Val Glu Asp  
 1024 GTT AAC CAG TGG ATT AAG CAG CCT CTC ATG GAT AAG AAC AGA ATA GAG GAG AGA TTG AAT TTA GTG GAA CCT TTT GTA GAA GAT  
  
 370 Ala Glu Leu Arg Gln Thr Leu Gln Glu Asp Leu Leu Arg Arg Phe Pro Asp Leu Asn Arg Leu Ala Lys Lys Phe Gln Arg Gln  
 1108 GCA GAA TTG AGG CAG ACT TTA CAA GAA GAT TTA CTT CGT CGA TTC CCA GAT CTT AAC CGA CTT GCC AAG AAG TTT CAA AGA CAA  
  
 398 Ala Ala Asn Leu Gln Asp Cys Tyr Arg Leu Tyr Gln Gly Ile Asn Gln Leu Pro Asn Val Ile Gln Ala Leu Glu Lys His Glu  
 1192 GCA GCA AAC TTA CAA GAT TGT TAC CGA CTC TAT CAG GGT ATA AAT CAA CTA CCT AAT GTT ATA CAG GCT CTG GAA AAA CAT GAA  
  
 426 Gly Lys His Gln Lys Leu Leu Ala Val Phe Val Thr Pro Leu Thr Asp Leu Arg Ser Asp Phe Ser Lys Phe Gln Glu Met  
 1276 GGA AAA CAC CAG AAA TTA TTG TTG GCA GTT TTT GTG ACT CCT CTT ACT GAT CTT CGT TCT GAC TTC TCC AAG TTT CAG GAA ATG  
  
 454 Ile Glu Thr Thr Leu Asp Met Asp Gln Val Glu Asn His Glu Phe Leu Val Lys Pro Ser Phe Asp Pro Asn Leu Ser Glu Leu  
 1360 ATA GAA ACA ACT TTA GAT ATG GAT CAG GTG GAA AAC CAT GAA TTC CTT GTA AAA CCT TCA TTT GAT CCT AAT CTC AGT GAA TTA  
  
 482 Arg Glu Ile Met Asn Asp Leu Glu Lys Lys Met Gln Ser Thr Leu Ile Ser Ala Ala Arg Asp Leu Gly Leu Asp Pro Gly Lys  
 1444 AGA GAA ATA ATG AAT GAC TTG GAA AAG AAG ATG CAG TCA ACA TTA ATA AGT GCA GCC AGA GAT CTT GGC TTG GAC CCT GGC AAA  
  
 510 Gln Ile Lys Leu Asp Ser Ser Ala Gln Phe Gly Tyr Tyr Phe Arg Val Thr Cys Lys Glu Glu Lys Val Leu Arg Asn Asn Lys  
 1528 CAG ATT AAA CTG GAT TCC AGT GCA CAG TTT GGA TAT TAC TTT CGT GTA ACC TGT AAG GAA GAA AAA GTC CTT CGT AAC AAT AAA  
  
 538 Asn Phe Ser Thr Val Asp Ile Gln Lys Asn Gly Val Lys Phe Thr Asn Ser Lys Leu Thr Ser Leu Asn Glu Glu Tyr Thr Lys  
 1612 AAC TTT AGT ACT GTA GAT ATC CAG AAG AAT GGT GTT AAA TTT ACC AAC AGC AAA TTG ACT TCT TTA AAT GAA GAG TAT ACC AAA  
  
 566 Asn Lys Thr Glu Tyr Glu Glu Ala Gln Asp Ala Ile Val Lys Glu Ile Val Asn Ile Ser Ser Gly Tyr Val Glu Pro Met Gln  
 1696 AAT AAA ACA GAA TAT GAA GAA GCC CAG GAT GCC ATT GTT AAA GAA ATT GTG AAT ATT TCT TCA GGC TAT GTA GAA CCA ATG CAG  
  
 594 Thr Leu Asn Asp Val Leu Ala Gln Leu Asp Ala Val Val Ser Phe Ala His Val Ser Asn Gly Ala Pro Val Pro Tyr Val Arg  
 1780 ACA CTC AAT GAT GTG TTA GCT CAG CTA GAT GCT GTT GTC AGC TTT GCT CAC GTG TCA AAT GGA CCA CCT GTT CCA TAT GTA CGA  
  
 622 Pro Ala Ile Leu Glu Lys Gly Gln Glu Arg Ile Ile Leu Lys Ala Ser Arg His Ala Cys Val Glu Val Gln Asp Glu Ile Ala  
 1864 CCA GCC ATT TTG GAG AAA GGA CAA GGA ACA ATT ATA TTA AAA GCA TCC AGG CAT GCT TGT GTT GAA GTT CAA GAT GAA ATT GCA

SUBSTITUTE SHEET (RULE 26)

## FIG. 5C

650 Phe Ile Pro Asn Asp Val Tyr Phe Glu Lys Asp Lys Gln Met Phe His Ile Ile Thr Gly Pro Asn Met Gly Gly Lys Ser Thr  
 1948 TTT ATT CCT AAT GAC GTA TAC TTT GAA AAA GAT AAA CAG ATG TTC CAC ATC ATT ACT GGC CCC AAT ATG GGA GGT AAA TCA ACA  
  
 678 Tyr Ile Arg Gln Thr Gly Val Ile Val Leu Met Ala Gln Ile Gly Cys Phe Val Pro Cys Glu Ser Ala Glu Val Ser Ile Val  
 2032 TAT ATT CGA CAA ACT GGG GTG ATA GTA CTC ATG GCC CAA ATT GGG TGT TTT GTG CCA TGT CAG TCA GCA GAA GTG TCC ATT GTG  
  
 706 Asp Cys Ile Leu Ala Arg Val Gly Ala Gly Asp Ser Gln Leu Lys Gly Val Ser Thr Phe Met Ala Glu Met Leu Glu Thr Ala  
 2116 GAC TGC ATC TTA GCC CGA GTA GGG GCT GGT GAC AGT CAA TTG AAA GGA GTC TCC ACG TTC ATG GCT GAA ATG TTG GAA ACT GCT  
  
 734 Ser Ile Leu Arg Ser Ala Thr Lys Asp Ser Leu Ile Ile Asp Glu Leu Gly Arg Gly Thr Ser Thr Tyr Asp Gly Phe Gly  
 2200 TCT ATC CTC AGG TCT GCA ACC AAA GAT TCA TTA ATA ATC ATA GAT CAA TTG GGA AGA GGA ACT TCT ACC TAC GAT GGA TTT GGG  
  
 762 Leu Ala Trp Ala Ile Ser Glu Tyr Ile Ala Thr Lys Ile Gly Ala Phe Cys Met Phe Ala Thr His Phe His Glu Leu Thr Ala  
 2284 TTA GCA TGG GCT ATA TCA GAA TAC ATT GCA ACA AAG ATT GGT GCT TTT TGC ATG TTT GCA ACC CAT TTT CAT GAA CTT ACT GCC  
  
 790 Leu Ala Asn Gln Ile Pro Thr Val Asn Asn Leu His Val Thr Ala Leu Thr Thr Glu Glu Thr Leu Thr Met Leu Tyr Gln Val  
 2368 TTG GCC AAT CAG ATA CCA ACT GTT AAT AAT CTA CAT GTC ACA GCA CTC ACC ACT GAA GAG ACC TTA ACT ATG CTT TAT CAG GTGG  
  
 818 Lys Lys Gly Val Cys Asp Gln Ser Phe Gly Ile His Val Ala Glu Leu Ala Asn Phe Pro Lys His Val Ile Glu Cys Ala Lys  
 2452 AAG AAA GGT GTC TGT GAT CAA AGT TTT GGG ATT CAT GTT GCA GAG CTT GCT AAT TTC CCT AAG CAT GTA ATA GAG TGT GCT AAA  
  
 846 Gln Lys Ala Leu Glu Leu Glu Glu Phe Gln Tyr Ile Gly Glu Ser Gln Gly Tyr Asp Ile Met Glu Pro Ala Ala Lys Lys Cys  
 2536 CAG AAA GCC CTG GAA CTT GAG GAG TTT CAG TAT ATT GGA GAA TCG CAA GGA TAT GAT ATC ATG GAA CCA GCA GCA AAG AAG TGC  
  
 874 Tyr Leu Glu Arg Glu Gln Gly Glu Lys Ile Ile Gln Glu Phe Leu Ser Lys Val Lys Gln Met Pro Phe Thr Glu Met Ser Glu  
 2620 TAT CTG GAA AGA GAG CAA GGT GAA AAA ATT ATT CAG GAG TTC CTG TCC AAG GTG AAA CAA ATG CCC TTT ACT GAA ATG TCA GAA  
  
 902 Glu Asn Ile Thr Ile Lys Leu Lys Gln Leu Lys Ala Glu Val Ile Ala Lys Asn Asn Ser Phe Val Asn Glu Ile Ile Ser Arg  
 2704 GAA AAC ATC ACA ATA AAG TTA AAA CAG CTA AAA GCT GAA GTA ATA GCA AAG AAT AAT AGC TTT GTA AAT GAA ATC ATT TCA CGA  
  
 930 Ile Lys Val Thr Thr  
 2788 ATA AAA GTT ACT ACG TGA AAA ATC CCA GTA ATG GAA TGA AGG TAA TAT TGA TAA GCT ATT GTC TGT AAT AGT TTT ATA TTG TTT  
  
 2872 TAT ATT AA

SUBSTITUTE SHEET (RULE 26)

## FIG. 6A

|       |  |     |
|-------|--|-----|
| hMSH2 | 11avqpkaetlql[ESAA]FVGFV[EFQ]GMP[EKPTT]TVRLFRGDEVTAH[EDALLAAREVEK]TGVikymgpagaknlqsvv1           | 80  |
| yMSH2 | 11sstrpelkfs[EVSE]ERN[EVK]YT[GGE]KPLK[TVRLV]DKGDYTVI[ESDAIFV]ADS[WH]TOS[VL]Kncqldpvtaknfhep      | 80  |
| hMSH2 | 11skmnfe[sv]kdl11[LV]qyrvevyknragkask[END]WYAYKASPGNLSQ[FE]DIEFGNNDSAS[IGVV]GVKMSAV[DE]QRQ       | 160 |
| yMSH2 | 11kryvtv[sl]qvlatt11[kl]cll[sl]gykveiy----[DKGMK]LIKASPGNIEQVNE[ELNMN]ID[SSII]IASLK[VQWNSQ]DNCI  | 156 |
| hMSH2 | 11VGVG[VDS]IQRKLG[CE]FPDNDQ[ESN]LEALL[IG]IPKECVL----PGGE[ACDN]GKLRQ[LIQ]RGSILL[TERK]KADFSTKD     | 236 |
| yMSH2 | 11IGVAFIDTAYKVG[GM]LDIVDNEVYSNLESF[LIQ]LGKCELVqd1tSNSNSNAEMQKVINVIDRC[GC]VTL[LLKN]SEFFSEKD       | 236 |
| hMSH2 | 11YQD[LR]RL[kgk]gegmnsav[LP]EMENQVAVSSLS[AVIK]ELELLSDSNFQ[FE]LTTTFDFS[OYMK]LDIAAVRALNLFQGS       | 316 |
| yMSH2 | 11VELD[IKLL]gddlals-----[LPQKY]SKLSMGACN[AL]G[V]IOLLSEQDQV[GK]VELVEHKLKEFWMKLDASAIKALNLFPGQ      | 311 |
| hMSH2 | 11VEDTTGSQS[AA]-----[LANK]ERTPQ[SOR]LVN[GM]IKOPLMDKMR[IEER]LNLV[EA]FVEDAELRQTLQED                | 379 |
| yMSH2 | 11PQNPFGSN[LA]Vsgftsagnsgkvtslfcl[LA]H[CKY]NAGVRLNENLKOPLTNIDE[INKR]HDDVDY[LI]DQIELROMLTSE       | 391 |
| hMSH2 | 11L[RRFP]DLNRLAKK[FQRQ]aANLQDCYEL[YGQ]INQ[LPNV]IOALEkhegkhqk-----[LL]LA[V]FV[PLT]DLRSDF[SK]FOEM  | 453 |
| yMSH2 | 11Y[PMI]PDIRRLTKKLNKR-GNLEDV[LI]YOF[SKRI]PEI[VQ]VFTsfleddsp[te]pvne[QVRS]VWLA[PLSHH]VEPL[SK]FEEM | 470 |
| hMSH2 | 11ETVLDMQDVENHE-FLAVRPS[EE]PNLSELREIMND[LEKK]QOSTLISAARDLG[DPGKQIK]DSSAQ[FGYY]FV[CKE]ERV         | 532 |

SUBSTITUTE SHEET (RULE 26)

# FIG. 6B

|       |                    |  |
|-------|--------------------|--|
| ymsh2 | 550                | VEITVLDLAYEENNeFMIRKVEFNEELGKIRSKLDTLRDEIHSIHLDSEADLGFDPKKLRLENHHLHGWCMLLRNDAKE      |
| hmsH2 | 612                | LRNNKNESTVDIQANCVKFTNSKLTSLNEEYTKNKTETEBAQDAIVKEIVNISSGVEPMQTLNDVLAOLDADVVSFAHVS     |
| ymsh2 | 630                | LRKHKKYIELSTVKAQIFESTKQKSLANETNIILOKEVDKQCSALVREININILTYTPVFEEKLSLVLAHLDDVLAFAHVS    |
| hmsH2 | 691                | NGAPVPYVRPailekgqg-RIIKASRRHACVEVODEIAEIPNDVYFEKDKQMEHTITGPNMGCKSTYIROTGVIVLMAOI     |
| ymsh2 | 710                | SYAPIPYIRPKlhpm dserRTHLISRRHPVLEMODDISEISNDVTLSECKGDELITGPNMGCKSTYIROTGVIVLMAOI     |
| hmsH2 | 771 <sup>910</sup> | GCEVPCESAEVSIVDCILARVGAGDSOLKGVSTFEMADMETASILRSATKDSLITIDELGRGTSTYDGEGLAWAISEYIA     |
| ymsh2 | 790                | GCEVPCEBAEIAIVDAILCRVGAGDSOLKGVSTFEMVEIETASILKNASKNSLTIIVDELGRGTSTYDGEGLAWAIAEHIA    |
| hmsH2 | 842                | TKIGAFCMFATHFHELTALANQIPTVNNLHVTA1tt-----EETLTMLYQVKKGVCCDOSEFGIHVAELANFPKHVIE       |
| ymsh2 | 870                | SKIGCFALFATHFHELTSELSEKLPNVKNMHVVAhieknlkeqkhdDEDITLLYKVPEGICDQSEFGIHVAEVDVQFPEKIVK  |
| hmsH2 | 922                | CAKQKALELEEFQYIGESqgydiTepaakkcyLeLeqqekiiqueflskvkmptfemseeniCiklkqJlkaevJlknnsf    |
| ymsh2 | 950                | MAKRAANELDDLKTNNEdlkkakJslqevnegniCJlkallkewirkvJeeqJlhdpskitJeeasqhkiJellraJlanepek |
| hmsH2 | 934                | vneiisrJkvJtt----  |
| ymsh2 | 966                | endhylefykspccyn   |

SUBSTITUTE SHEET (RULE 26)

10/10

A C G T



FIG. 7A



FIG. 7B

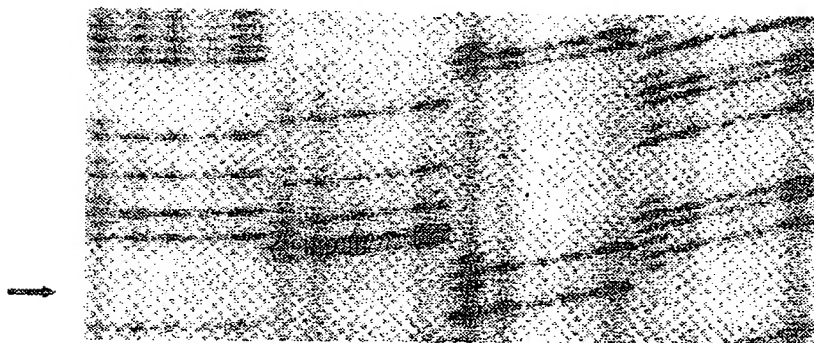


FIG. 7C

1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5

SUBSTITUTE SHEET (RULE 26)





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|   |           |   |
|---|-----------|---|
| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12N 15/12, C12Q 1/68, A01K 67/00,</b><br><b>A61K 48/00, C07K 14/47</b>  | <b>A3</b> | <b>(11) International Publication Number:</b> <b>WO 95/15381</b><br><b>(43) International Publication Date:</b> <b>8 June 1995 (08.06.95)</b>   |
| <b>(21) International Application Number:</b> <b>PCT/US94/13805</b><br><b>(22) International Filing Date:</b> <b>2 December 1994 (02.12.94)</b><br><br><b>(30) Priority Data:</b><br><b>160,295</b> <b>2 December 1993 (02.12.93)</b> <b>US</b><br><br><b>(71) Applicant:</b> <b>THE JOHNS HOPKINS UNIVERSITY [US/US];</b><br><b>720 Rutland Avenue, Baltimore, MD 21205 (US).</b><br><br><b>(71)(72) Applicant and Inventor:</b> <b>DE LA CHAPELLE, Albert</b><br><b>[FI/FI]; Bregmansgatan 11, FIN-00140 Helsingfors (FI).</b><br><br><b>(72) Inventors:</b> <b>VOGELSTEIN, Bert; 3700 Breton Way, Baltimore,</b><br><b>MD 21208 (US). KINZLER, Kenneth, W.; 1348 Halstead</b><br><b>Road, Baltimore, MD 21234 (US).</b><br><br><b>(74) Agents:</b> <b>KAGAN, Sarah, A. et al.; Banner, Birch, McKie &amp;</b><br><b>Beckett, 11th floor, 1001 G Street, N.W., Washington, DC</b><br><b>20001 (US).</b> |           | <b>(81) Designated States:</b> <b>CA, JP, European patent (AT, BE, CH, DE,</b><br><b>DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</b><br><br><b>Published</b><br><i>With international search report.</i><br><i>Before the expiration of the time limit for amending the</i><br><i>claims and to be republished in the event of the receipt of</i><br><i>amendments.</i><br><br><b>(88) Date of publication of the international search report:</b><br><b>22 June 1995 (22.06.95)</b> |
| <b>(54) Title:</b> <b>HUMAN MUTATOR GENE hMSH2 AND HEREDITARY NON-POLYPOSIS COLORECTAL CANCER</b><br><br><b>(57) Abstract</b><br><br><p>The human <i>MSH2</i> gene, responsible for hereditary non-polyposis colorectal cancer, was identified by virtue of its homology to the <i>MutS</i> class of genes, which are involved in DNA mismatch repair. The sequence of cDNA clones of the human gene are provided, and the sequence of the gene can be used to demonstrate the existence of germ line mutations in hereditary non-polyposis colorectal cancer (HNPCC) kindreds, as well as in replication error<sup>+</sup> (RER<sup>+</sup>) tumor cells.</p>  |           |   |

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |                                       |    |                          |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria                  | GB | United Kingdom                        | MR | Mauritania               |
| AU | Australia                | GE | Georgia                               | MW | Malawi                   |
| BB | Barbados                 | GN | Guinea                                | NE | Niger                    |
| BE | Belgium                  | GR | Greece                                | NL | Netherlands              |
| BF | Burkina Faso             | HU | Hungary                               | NO | Norway                   |
| BG | Bulgaria                 | IE | Ireland                               | NZ | New Zealand              |
| BJ | Benin                    | IT | Italy                                 | PL | Poland                   |
| BR | Brazil                   | JP | Japan                                 | PT | Portugal                 |
| BY | Belarus                  | KE | Kenya                                 | RO | Romania                  |
| CA | Canada                   | KG | Kyrgyzstan                            | RU | Russian Federation       |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan                    |
| CG | Congo                    | KR | Republic of Korea                     | SE | Sweden                   |
| CH | Switzerland              | KZ | Kazakhstan                            | SI | Slovenia                 |
| CI | Côte d'Ivoire            | LI | Liechtenstein                         | SK | Slovakia                 |
| CM | Cameroon                 | LK | Sri Lanka                             | SN | Senegal                  |
| CN | China                    | LU | Luxembourg                            | TD | Chad                     |
| CS | Czechoslovakia           | LV | Latvia                                | TG | Togo                     |
| CZ | Czech Republic           | MC | Monaco                                | TJ | Tajikistan               |
| DE | Germany                  | MD | Republic of Moldova                   | TT | Trinidad and Tobago      |
| DK | Denmark                  | MG | Madagascar                            | UA | Ukraine                  |
| ES | Spain                    | ML | Mali                                  | US | United States of America |
| FI | Finland                  | MN | Mongolia                              | UZ | Uzbekistan               |
| FR | France                   |    |                                       | VN | Viet Nam                 |
| GA | Gabon                    |    |                                       |    |                          |



|   |   |   |
|---|---|---|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC 6 C12N15/12 A61K48/00 C12Q1/68 C07K14/47 A01K67/00  |   |   |
| According to International Patent Classification (IPC) or to both national classification and IPC   |   |   |
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC 6 C07K  |   |   |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched   |   |   |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  |   |   |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |   |   |
| Category *  | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.   |
| A   | SCIENCE,<br>vol.260, 7 May 1993<br>pages 810 - 812<br>PELTOMAEKI, P. ET AL. 'Genetic mapping of<br>a locus predisposing to human colorectal<br>cancer'<br>cited in the application<br>---   | 1   |
| A   | NATURE,<br>vol.363, 10 June 1993<br>pages 558 - 561<br>IONOV, Y. ET AL. 'Ubiquitous somatic<br>mutations in simple repeated sequences<br>reveal a new mechanism for colonic<br>carcinogenesis'<br>cited in the application<br>---<br><div style="text-align: center;">-/-</div> | 1   |
| <div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.</span> <span><input type="checkbox"/> Patent family members are listed in annex.</span> </div>  |   |   |
| <div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents:</p> <p>'A' document defining the general state of the art which is not considered to be of particular relevance</p> <p>'E' earlier document but published on or after the international filing date</p> <p>'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>'O' document referring to an oral disclosure, use, exhibition or other means</p> <p>'P' document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>'&amp;' document member of the same patent family</p> </div> </div> |   |   |
| Date of the actual completion of the international search<br><br><div style="text-align: center;">2 May 1995</div>  |   | Date of mailing of the international search report<br><br><div style="text-align: center;">24. 05. 95</div> |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+ 31-70) 340-2040, Tx. 31 651 cpo nl,<br>Fax (+ 31-70) 340-3016   |   | Authorized officer<br><br><div style="text-align: center;">Alt, G</div>                                     |

Form PCT/ISA/210 (second sheet) (July 1992)

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|--|---|-----------------------|
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| A  | NATURE,<br>vol.365, 16 September 1993<br>pages 274 - 276<br>STRAND, M. ET AL. 'Destabilization of<br>tracts of simple repetitive DNA in yeast<br>by mutations affecting DNA mismatch<br>repair'<br>cited in the application<br>---- | 1                     |
| P,X  | CELL,<br>vol.75, 3 December 1993<br>pages 1027 - 1038<br>FISHEL, R. ET AL. 'The human mutator gene<br>homolog MSH2 and its association with<br>hereditary nonpolyposis colon cancer'<br>see Figure 1<br>----                        | 1-26                  |
| P,X  | CELL,<br>vol.75, 17 December 1993<br>pages 1215 - 1225<br>LEACH, F.S. ET AL. 'Mutations of a mutS<br>homolog in hereditary nonpolyposis<br>colorectal cancer'<br>see Figure 5<br>----   | 1-26                  |
| P,A  | CELL,<br>vol.75, 17 December 1993<br>pages 1227 - 1236<br>PARSONS, R. ET AL. 'Hypermutability and<br>mismatch repair deficiency in RER+ tumor<br>cells'<br>-----  | 1                     |